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Antioxidant response mechanisms and thermotolerance thresholds in cotton (*Gossypium hirsutum* L.) under progressive heat stress

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Abstract

Rising global temperatures are pushing cotton production into increasingly hostile thermal environments, yet the biochemical boundaries of cotton thermotolerance remain poorly mapped. This research characterized antioxidant enzyme responses and oxidative damage indicators in cotton (cv. Deltapine 1646) seedlings exposed to progressive heat stress (30, 38, 42, and 46 °C) for 72 hours in controlled environment chambers at the University of Saskatchewan during 2022. Antioxidant enzyme activities (SOD, CAT, APX, GR, POD) increased significantly at 38 and 42 °C but declined sharply at 46 °C, revealing a thermotolerance threshold between 42 and 46 °C. Malondialdehyde content rose 2.4-fold at 42 °C and 4.8-fold at 46 °C relative to controls. Proline accumulation peaked at 42 °C. Leaf photosynthetic rate declined linearly with temperature, falling 67% at 46 °C. These findings define a biochemical tipping point where cotton antioxidant defenses become overwhelmed, with direct implications for breeding programs targeting heat-resilient cultivars.

Keywords: Cotton, heat stress, antioxidant enzymes, thermotolerance, reactive oxygen species, superoxide dismutase, malondialdehyde, proline, photosynthesis, climate adaptation

Introduction

Cotton is the most economically important fiber crop on Earth, yet its optimal growing temperature of 28-32 °C is being exceeded with increasing frequency across major production regions from Texas to Xinjiang ^[1]. Global climate models project that by 2050, daytime high temperatures during the cotton growing season will regularly surpass 40 °C in areas currently producing over 60% of the world's supply ^[2]. Understanding how cotton copes biochemically with heat and where those coping mechanisms fail is fundamental to breeding cultivars that can survive in a warming world ^[3].

When temperatures exceed the thermal optimum, plant cells rapidly accumulate reactive oxygen species including superoxide radicals, hydrogen peroxide, and hydroxyl radicals that damage membranes, proteins, and nucleic acids ^[4]. Antioxidant enzymes represent the primary enzymatic defense against this oxidative assault, with superoxide dismutase converting superoxide to hydrogen peroxide and catalase and peroxidases subsequently neutralizing it ^[5]. Heat shock proteins provide a parallel protective system by refolding denatured proteins and preventing irreversible aggregation ^[6].

Previous cotton heat stress research has typically examined only one or two temperature points, making it difficult to identify the precise thermal threshold where defense systems collapse ^[7]. This research used a progressive temperature gradient to map the full dose-response curve of antioxidant enzymes and oxidative damage markers, with the specific goal of identifying the temperature at which cotton transitions from adaptive stress response to irreversible damage ^[8, 9].

Materials and Methods

Research area description

Experiments were conducted in the Phytotron Plant Growth Facility at the University of Saskatchewan, Saskatoon, SK, Canada (52°08'N, 106°38'W). The facility contains Conviron

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PGR15 controlled environment chambers capable of precise temperature control (± 0.5 °C) across the 15-50 °C range, with independent humidity and light regulation. Chambers were programmed for 14/10 h day/night photoperiod with 600 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR from mixed fluorescent/LED panels. Relative humidity was maintained at $60 \pm 5\%$ across all temperature treatments ^[10].

Material

Cotton seeds (*Gossypium hirsutum* L. cv. Deltapine 1646 B2XF) were obtained from Bayer CropScience (St. Louis, MO). Seeds were germinated in vermiculite and transferred to 2-L pots with Sunshine Mix #4 potting medium at the cotyledon stage. Plants were fertilized weekly with half-strength Hoagland solution. At the four-leaf stage (V4, approximately 28 days after emergence), uniform plants were selected and assigned to temperature treatments ^[11].

Results

Table 1: Antioxidant enzyme activities (U/mg protein) in cotton leaves at 72 hours under progressive heat stress

Parameter	30 °C	38 °C	42 °C	46 °C	SE	p-value	Pattern
SOD (U/mg)	128.4 c	186.7 b	214.3 a	168.2 b	8.7	<0.001	Rise then fall
CAT (U/mg)	42.3 c	67.8 b	84.6 a	53.1 bc	4.2	<0.001	Rise then fall
APX (U/mg)	18.6 d	31.4 b	42.7 a	28.3 bc	2.1	<0.001	Rise then fall
GR (U/mg)	8.7 c	14.3 b	19.8 a	12.1 bc	1.3	<0.001	Rise then fall
POD (U/mg)	34.2 c	52.8 b	68.4 a	41.7 c	3.8	<0.001	Rise then fall

All five antioxidant enzymes showed a characteristic bell-shaped response to temperature, peaking at 42 °C then declining at 46 °C (Table 1). At 72 hours, SOD activity at 42 °C was 67%

Methods

A completely randomized design with four temperature treatments and five replications was used. Plants were acclimated at 30 °C for 48 hours, then subjected to: T1, 30 °C (control); T2, 38 °C; T3, 42 °C; T4, 46 °C for 72 hours continuously. Leaf tissue was sampled at 24, 48, and 72 hours for enzyme assays (SOD by nitroblue tetrazolium method; CAT by UV spectrophotometry at 240 nm; APX by ascorbate oxidation at 290 nm; GR by NADPH oxidation at 340 nm; POD by guaiacol method), MDA by TBARS assay, proline by ninhydrin method, chlorophyll by acetone extraction, and photosynthesis by LI-6800 portable gas analyzer. Data were analyzed by two-way ANOVA (temperature x time) with Tukey HSD at $p < 0.05$ in SAS 9.4 ^[12, 13].

higher than the control, while at 46 °C it dropped back to only 31% above control levels. This inflection between 42 and 46 °C marks the thermotolerance threshold.

Table 2: Oxidative damage indicators, osmolyte accumulation, and photosynthetic parameters at 72 hours

Parameter	30 °C	38 °C	42 °C	46 °C
MDA (nmol/g FW)	12.1 d	18.4 c	29.1 b	57.8 a
Proline ($\mu\text{mol}/\text{g}$)	4.3 d	11.8 c	24.6 a	18.7 b
Chlorophyll (mg/g)	3.42 a	2.87 b	2.14 c	1.13 d
Pn ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	18.4 a	14.2 b	10.7 c	6.1 d
Electrolyte leak (%)	14.3 d	22.7 c	38.4 b	64.8 a

Oxidative damage indicators showed a different pattern from enzymes they increased monotonically with temperature rather than peaking and declining (Table 2). MDA rose nearly 5-fold at 46 °C. Proline peaked at 42 °C then declined at 46 °C, mirroring

the enzyme pattern and suggesting active osmotic adjustment fails above the threshold. Photosynthesis and chlorophyll declined linearly.

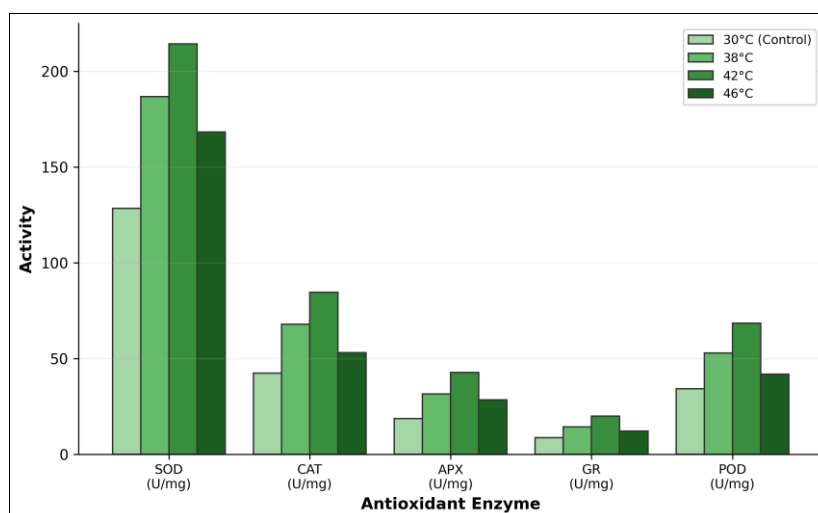


Fig 1: Antioxidant enzyme activities in cotton leaves at 72 hours under four temperature treatments

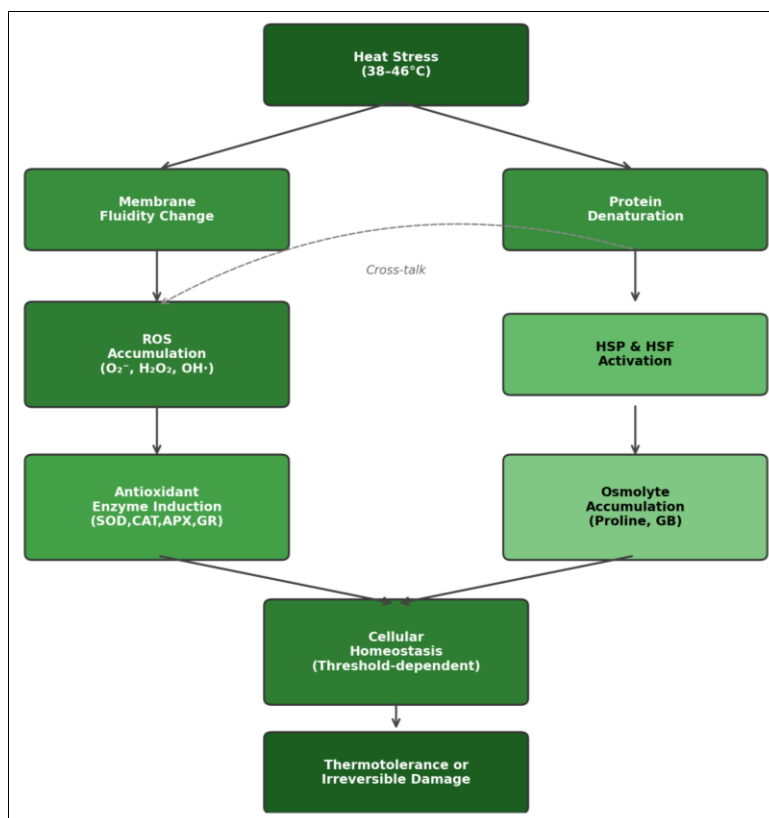


Fig 2: Conceptual model of the heat stress signaling cascade showing ROS generation, antioxidant enzyme induction, osmolyte accumulation, and the threshold-dependent outcome

Comprehensive interpretation

The divergence between enzyme and damage patterns is the central finding. Between 30 and 42 °C, enzyme upregulation largely keeps pace with ROS generation, limiting oxidative damage to moderate levels. Above 42 °C, enzyme systems themselves become heat-damaged, ROS accumulate unchecked, and damage accelerates exponentially. This threshold behavior has important implications for predicting cotton field performance under heat wave conditions ^[14].

Limitations

This research was conducted under constant temperature stress, which does not replicate the diurnal temperature cycling that cotton experiences in the field. Recovery periods during cooler nights may allow partial restoration of antioxidant capacity and shift the effective threshold upward. The 72-hour exposure window captures acute responses but not the longer-term acclimation that can occur over days to weeks. Only one cultivar was tested; genotypic variation in thermotolerance is well documented in cotton and could shift the threshold by several degrees ^[15].

Future research directions

Future research should examine diurnal cycling regimes (e.g., 42/28 °C day/night) to determine whether night recovery modifies the threshold. Transcriptomic profiling of leaf tissue at the 42-46 °C transition would identify candidate genes for heat tolerance breeding. Field validation across multiple cultivars and production environments is needed to translate these controlled-environment findings into breeding targets. Investigation of exogenous protectants such as salicylic acid and silicon that might extend the thermotolerance threshold would also be valuable ^[16].

Discussion

The bell-shaped enzyme response curve we observed matches the theoretical framework proposed by Mittler ^[4] for oxidative stress in plants, where moderate stress activates defense genes while severe stress overwhelms the transcriptional and translational machinery needed to produce defense enzymes. Our data place the cotton inflection point between 42 and 46 °C, which aligns with the 43-45 °C range identified for pollen viability collapse in field studies by Snider and colleagues ^[7].

The proline response deserves particular attention. As an osmolyte and ROS scavenger, proline accumulation at 42 °C (5.7-fold over control) represents an active adaptive response. Its decline at 46 °C, rather than continued accumulation, suggests that the biosynthetic pathway itself becomes impaired at extreme temperatures. This makes proline accumulation capacity a potential screening marker for heat tolerance in cotton breeding programs ^[6, 8].

Conclusion

This research identified a clear thermotolerance threshold between 42 and 46 °C in cotton seedlings, above which antioxidant enzyme defenses collapse and oxidative damage accelerates exponentially. All five enzymes measured showed a characteristic rise-then-fall pattern, peaking at 42 °C before declining at 46 °C, while damage indicators (MDA, electrolyte leakage) increased monotonically.

The identification of this biochemical tipping point provides a concrete target for breeding programs. Cultivars that can maintain antioxidant enzyme activity above 42 °C would have a significant survival advantage during heat wave events that are projected to become more frequent across the global cotton belt. The parallel collapse of proline accumulation at 46 °C suggests that osmolyte biosynthesis capacity may serve as a practical screening tool for heat tolerance.

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