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A silicon-based formulation enhances antioxidant defense and biochemical stability in heat-stressed cotton

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Abstract

Global temperature rise increasingly exposes crops to heat stress, leading to oxidative damage and reduced productivity. The present study investigated the effects of seed priming with a silicon-based formulation on antioxidant defense mechanisms and biochemical responses in two cotton (*Gossypium hirsutum* L.) cultivars (**Bukhara-8 and Bukhara-102) under short-term heat stress (45 °C for 6 h). Seeds were primed with the commercial silicon-based formulation Aminosid-Silicon (170 mg Si L^{-1}) and evaluated using a 2 × 2 × 3 factorial experimental design. Heat stress increased oxidative damage, whereas treatment with the silicon-based formulation enhanced antioxidant enzyme activities. The greatest response was observed for POD activity, which increased from 22.0 to 29.6 U mg^{-1} protein in Bukhara-102, while H_2O_2 content decreased from 158.2 to 119.4 nmol g^{-1} FW under heat stress**. The silicon-based formulation also promoted proline accumulation, and Pearson correlation analysis revealed coordinated relationships among antioxidant enzymes, oxidative stress markers, and proline. Overall, the results indicate that seed priming with the silicon-based formulation enhanced biochemical protection against acute heat stress by strengthening antioxidant defense and reducing oxidative damage in cotton seedlings.

Keywords: Hyperthermia, Lipid peroxidation, Proline metabolism, Reactive oxygen species, Silicon priming.

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Introduction

Global climate change has resulted in a pronounced increase in average air temperatures, posing a serious threat to agricultural productivity worldwide (Yuan et al., 2024). In arid and semi-arid regions, where summer temperatures frequently exceed 40–45°C, short-term hyperthermia events constitute one of the most critical abiotic stress factors limiting crop performance (Rajanna et al., 2023). Elevated temperatures disrupt cellular metabolism and promote excessive generation of reactive oxygen species (ROS), resulting in oxidative stress, membrane damage, and reduced plant growth and productivity (Alam et al., 2025). The over accumulation of ROS induces oxidative stress, leading to lipid peroxidation, protein denaturation, nucleic acid damage, and ultimately reduced plant growth and yield. Therefore, elucidating the biochemical mechanisms underlying heat stress tolerance is essential for enhancing crop resilience under ongoing climate warming (Hasanuzzaman et al., 2020).

Cotton (*Gossypium hirsutum* L.), although classified as a thermophilic crop, exhibits optimal growth within a relatively narrow temperature range (Li et al., 2020). Exposure to temperatures beyond this threshold disrupts cellular homeostasis and redox balance. Proline acts as an osmoprotectant, stabilizes proteins and membranes, scavenges reactive oxygen species, and contributes to cellular redox homeostasis. Its accumulation is a common adaptive response to heat stress, although severe hyperthermia may disrupt proline metabolism (Carillo, 2025; (Deuschle et al., 2004).

Malondialdehyde (MDA), a stable end-product of lipid peroxidation, is widely used as an indicator of oxidative membrane damage under heat stress (Akbari et al., 2023; Sharma et al., 2012). Accordingly, proline reflects adaptive protection, whereas MDA indicates oxidative injury, and together these markers provide valuable insight into plant stress tolerance mechanisms (Karagüzel, 2026).

In recent years, silicon (Si) has been recognized as a beneficial element that enhances plant resistance to various abiotic stresses (Mamasolieva et al., 2024). Although not considered essential for plant growth, silicon has been reported to improve membrane stability, reinforce cell wall structure, modulate antioxidant defense systems (including superoxide dismutase, catalase, and ascorbate peroxidase activities), and reduce ROS accumulation (Hasanuzzaman et al., 2018). Emerging evidence further suggests that silicon may regulate proline metabolism and attenuate lipid peroxidation under stress conditions (Farouk et al., 2020). However, despite extensive studies

on heat stress physiology in cotton and silicon-mediated stress mitigation in other crops, the interactive effects of silicon on proline dynamics and lipid peroxidation under short-term hyperthermia in arid-adapted cotton cultivars remain insufficiently explored (Luqman et al., 2025). Therefore, the objective of the present study was to evaluate changes in proline and malondialdehyde concentrations in cotton cultivars exposed to short-term hyperthermia and to determine the role of the silicon-based formulation in modulating oxidative stress responses. We hypothesized that hyperthermia would enhance MDA accumulation and alter proline metabolism in a cultivar-dependent manner, whereas seed priming with the silicon-based formulation would promote proline accumulation, reduce lipid peroxidation, and enhance biochemical protection against heat-induced oxidative stress.

Material and methods

Plant material and treatments

Two upland cotton (*Gossypium hirsutum* L.) cultivars, Bukhoro-8 and Bukhoro-102, which are widely cultivated under irrigated conditions in Uzbekistan and differ in their responses to environmental stresses, were used in this study. The experiment was conducted under controlled laboratory conditions using a programmable growth chamber. The experimental design consisted of four treatment combinations: (i) untreated seedlings maintained under normal temperature (control), (ii) silicon-treated seedlings maintained under normal temperature, (iii) untreated seedlings exposed to heat stress, and (iv) silicon-treated seedlings exposed to heat stress.

For each biological replicate, 50 seeds were used. Seeds were delinted with concentrated sulfuric acid and thoroughly rinsed under running tap water for 15 min before being soaked in distilled water for 12 h. Silicon seed priming was performed by soaking the seeds for 24 h in an aqueous solution of the commercial product Aminosid-Silicon (17% Si and 9% N; Registration Certificate No. 11.298, issued on 14 May 2021), **providing 170 mg Si L^{-1}. The selected silicon concentration (170 mg Si L^{-1}) was based on the manufacturer's recommended application rate for agricultural use. This concentration is currently applied under field conditions and was selected to ensure that the experimental treatment reflected practical agronomic practice.** Control seeds were soaked in distilled water under identical conditions throughout the experiment. The experiment was designed to evaluate the biological effectiveness of the

commercial silicon-based formulation under practical application conditions rather than to isolate the physiological effects of its individual components.

Following treatment, the seeds were immediately subjected to germination using the paper-roll method in the programmable growth chamber maintained at 30 ± 1 °C in complete darkness for 7 days, with a relative humidity of 75–80%.

Heat stress was imposed by gradually increasing the chamber temperature from 30°C to 45 °C at a rate of 1°C every 10 min, followed by exposure to 45 °C for 6 h while maintaining 75–80% relative humidity. The heat stress regime was established according to the protocol described by Kolupaev et al. (2023) **for the controlled induction of heat stress and oxidative responses in seedlings. After heat treatment, seedlings assigned to the recovery treatment were transferred back** to 30 °C for 24 h before biochemical analyses. A 24-h recovery period **was used to evaluate early post-stress biochemical responses before substantial secondary developmental changes occurred.**

Biochemical analyses

Biochemical analyses were performed using whole 7-day-old cotton seedlings collected immediately after completion of the heat stress treatment or after the 24-h recovery period, depending on the experimental treatment.

Hydrogen peroxide (H_2O_2) content was determined spectrophotometrically according to the potassium iodide method described by Velikova et al. (2000). Lipid peroxidation was estimated by measuring malondialdehyde (MDA) concentration using the thiobarbituric acid (TBA) assay as described by Heath and Packer (1968). Superoxide dismutase (SOD) activity was determined based on inhibition of nitroblue tetrazolium reduction following the method of Beauchamp and Fridovich (1971). Peroxidase (POD) activity was assayed according to Chance and Maehly (1955). Catalase (CAT) activity was determined following the dichromate–acetic acid method described by Sinha (1972). Total soluble protein concentration was determined according to Lowry et al. (1951). Free proline content was measured using the acid ninhydrin method described by Bates et al. (1973).

Statistical analysis

The experiment followed a 2 × 2 × 3 factorial arrangement in a completely randomized design with three biological replicates (50 seedlings per replicate). The three experimental factors were cotton cultivar (Bukhara-8 and Bukhara-102), silicon-based formulation (with or without Aminosid-Silicon), and temperature stage (before heat stress, after 6 h at 45 °C, and after 24 h of recovery at 30°C following heat stress). Data were analyzed using GraphPad Prism software (version 8.0.1; GraphPad Software, San Diego, CA, USA). A three-way analysis of variance (ANOVA) was performed to evaluate the main effects of cultivar, silicon-based formulation, and temperature regime, as well as all possible interaction effects among these factors. When significant differences were detected, treatment means were compared using Tukey's honestly significant difference (HSD) test at $P < 0.05$. Pearson correlation analysis was performed using PAST software (version 4.03; Hammer et al., 2001) based on replicate-level data (n = 36), and correlation coefficients were considered statistically significant at $P < 0.05$. Results are presented as the mean ± standard error (SE) of three independent biological replicates (n = 3).

Results

Effect of silicon on growth inhibition under heat stress

Heat stress significantly affected seedling growth in both cotton cultivars (Table 1). Silicon-based formulation increased growth inhibition in both shoots and roots compared with untreated controls. In shoots, growth inhibition increased from 74.2% to 79.1% in Bukhara-8 and from 68.9% to 78.4% in Bukhara-102. Root growth exhibited a similar response, with consistently greater inhibition than shoots following silicon-based formulation. Significant differences between silicon-treated and untreated plants within each cultivar were confirmed by Tukey's HSD test ($P < 0.05$).

Factorial ANOVA confirmed that silicon-based formulation significantly affected growth inhibition ($P = 0.0079$), whereas cultivar had no significant main effect ($P = 0.729$). Growth inhibition also differed significantly between shoots and roots ($P < 0.0001$), while none of the interaction effects among cultivar, organ, and silicon-based formulation were statistically significant (Supplementary Table S1).

Effect of silicon application on antioxidant enzyme activities under heat stress

Silicon-based formulation significantly influenced antioxidant enzyme activities in both cultivars (Table 2). Under heat stress and recovery conditions, silicon-based formulation enhanced the activities of SOD, CAT, and POD. The response was most pronounced in Bukhara-102, where SOD activity increased from 16.9 to 19.4 U mg^{-1} protein, while POD activity increased from 22.0 to 29.6 U mg^{-1} protein. CAT activity also increased in both cultivars following silicon application, although to a lesser extent than POD.

Three-way factorial ANOVA confirmed significant main effects of silicon-based formulation and temperature stage on antioxidant enzyme activities ($P < 0.05$), whereas interaction effects varied among enzymes. A significant three-way interaction was detected only for SOD, while CAT and POD were primarily influenced by the main effects of silicon-based formulation and temperature (Supplementary Table S1).

Effect of silicon application on oxidative stress markers and proline accumulation under heat stress

Heat stress significantly increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in both cultivars (Table 3). Silicon-based formulation reduced H_2O_2 accumulation under both heat stress and recovery conditions, with the greatest reduction observed in Bukhara-102, where H_2O_2 decreased to 119.4 nmol g^{-1} FW during heat stress and 117.1 nmol g^{-1} FW during recovery. MDA content showed a similar response, with silicon-based formulation plants exhibiting lower lipid peroxidation than untreated controls. The largest reduction in MDA was observed in Bukhara-102, where the value declined from 14.7 to 11.3 nmol g^{-1} FW following recovery. Proline content increased under heat stress in both cultivars and was further enhanced by silicon-based formulation, reaching its highest level during the recovery stage.

Three-way factorial ANOVA demonstrated significant effects of silicon-based formulation and temperature stage on H_2O_2, MDA, and proline content. Significant silicon × temperature interactions for H_2O_2 and MDA indicated that the effectiveness of silicon depended on the stage of heat stress and recovery, whereas no significant interaction effects involving temperature were detected for proline (Supplementary Table S1).

182 **Correlation analysis**

183 **Pearson correlation analysis revealed significant relationships among antioxidant enzymes,**
184 **oxidative stress markers, and proline content (Fig. 1). Positive correlations were observed**
185 **among the antioxidant enzymes, with SOD being positively correlated with CAT (r = 0.68)**
186 **and POD (r = 0.72). SOD was also positively associated with proline content (r = 0.74), while**
187 **CAT and POD showed positive correlations with proline (r = 0.42 and r = 0.63, respectively).**
188 **In contrast, H_2O_2 exhibited negative correlations with SOD (r = −0.71), CAT (r = −0.64),**
189 **POD (r = −0.44), and proline (r = −0.55). H_2O_2 was positively correlated with MDA (r = 0.36),**
190 **whereas proline showed a moderate negative correlation with MDA (r = −0.45). Weak**
191 **correlations were observed between MDA and the antioxidant enzymes, whereas proline**
192 **showed a moderate negative correlation with MDA (r = −0.45), indicating that greater**
193 **proline accumulation was associated with lower lipid peroxidation. The positive associations**
194 **among antioxidant enzymes and proline, together with their negative correlations with H_2O_2,**
195 **suggest that enhanced antioxidant capacity is associated with reduced oxidative stress in**
196 **cotton seedlings. These relationships are consistent with the factorial ANOVA results**
197 **(Supplementary Table S1), which identified temperature stage as the primary source of**
198 **variation, while silicon-based formulation further strengthened the antioxidant defense**
199 **response. Complete ANOVA statistics, including degrees of freedom (df), F-values, and exact**
200 **P-values for all main effects and interaction terms, are provided in Supplementary Table S1.**

201

202 **Discussion**

203 Short-term hyperthermia markedly inhibited seedling growth in both cotton cultivars, confirming
204 that elevated temperature severely impairs early developmental processes. Growth inhibition
205 observed in shoots and roots indicates that thermal stress disrupts cellular metabolism and
206 elongation processes. Similar reductions in seedling growth under high temperature have been
207 documented in cotton and other crops, where heat stress interferes with cell division, membrane
208 integrity, and enzymatic activity (Kolupaev et al., 2023; Luqman et al., 2025).

209 **Although seedlings treated with the silicon-based formulation exhibited slightly greater**
210 **growth inhibition than untreated seedlings under acute heat stress, this response occurred**
211 **concurrently with enhanced antioxidant defense observed in the present study, including**
212 **increased activities of SOD, CAT, and POD together with reduced H_2O_2 and MDA**

accumulation. These biochemical responses indicate that the silicon-based formulation enhanced antioxidant defense and reduced oxidative damage at the cellular level despite the absence of an immediate improvement in seedling growth.

This apparent divergence between biochemical protection and morphological performance may reflect a transient shift in resource allocation toward cellular defense rather than growth during acute heat stress, a response that is consistent with the growth–defense trade-off described in plants (Hu et al., 2026). Consequently, metabolic resources may have been preferentially allocated to cellular defense rather than shoot and root elongation during acute heat stress. However, because biomass partitioning, carbon allocation, and post-stress growth recovery were not evaluated in the present study, this interpretation should be regarded as a plausible explanation rather than direct experimental evidence. This interpretation is supported by previous studies showing that silicon-induced activation of antioxidant metabolism may precede improvements in plant performance during later developmental stages under stress conditions (Saidova et al., 2026). Consistent with these observations, the present findings suggest that treatment with the silicon-based formulation promoted biochemical protection that may precede measurable improvements in seedling growth during acute heat stress.

The apparent discrepancy between the reduced H_2O_2 and MDA accumulation and the absence of immediate improvements in shoot and root growth is more likely attributable to the short duration of heat stress exposure (6 h) and the early seedling stage evaluated in the present study than to any adverse effect of the silicon-based formulation itself. Biochemical responses to stress generally occur earlier than measurable morphological changes, and the enhanced antioxidant protection observed here may require a longer recovery period before being translated into improved growth. This interpretation is further supported by our previous field study using the same Aminosid-Silicon formulation, in which enhanced antioxidant defense was accompanied by improved plant growth and cotton productivity under prolonged stress conditions (Saidova et al., 2026). Moreover, numerous recent studies have demonstrated that silicon functions as a beneficial element by enhancing tolerance to abiotic and biotic stresses, improving antioxidant defense, and promoting plant growth rather than inducing stress (Ahmed et al., 2023; Mamasolieva et al., 2024; Tayade et al., 2022). Therefore, the present findings should be interpreted as reflecting a temporal difference

between early biochemical protection and later morphological responses rather than evidence of silicon-induced stress.

Although seedlings treated with the silicon-based formulation did not exhibit immediate morphological recovery, the enhanced antioxidant defense and reduced oxidative damage observed in the present study indicate that silicon-based formulation improved cellular protection under heat stress. Accordingly, the findings of the present study should be interpreted as evidence of enhanced biochemical protection during acute heat stress rather than as direct evidence of improved seedling growth or whole-plant heat tolerance. Nevertheless, because the present study was limited to the early seedling stage under short-term heat stress, further physiological and long-term investigations are required to determine whether these early biochemical responses are subsequently translated into improved physiological performance, plant growth, and heat tolerance at later developmental stages under greenhouse and field conditions.

Hyperthermia induced pronounced oxidative stress, as evidenced by increased H_2O_2 and MDA accumulation. Elevated temperature is known to enhance ROS generation through disruption of electron transport chains and metabolic imbalance (Velikova et al., 2000), lipid peroxidation, and membrane damage in cotton seedlings (Zameer et al., 2022). **Application of the silicon-based formulation significantly enhanced antioxidant enzyme activities, including SOD, CAT, and POD. The coordinated stimulation of these enzymes suggests activation of an integrated ROS-scavenging system. Similar enhancement of antioxidant defense under heat stress has also been reported in cotton, where increased SOD, CAT, and POD activities were associated with improved oxidative stress tolerance** (Khan et al., 2026; Zhanassova et al., 2021)**, while silicon-induced activation of antioxidant metabolism has been demonstrated in several crop species** (Wang et al., 2021)**. The marked increase in POD activity observed in the present study indicates that hydrogen peroxide detoxification plays a central role in silicon-mediated stress mitigation. However, the factorial ANOVA demonstrated that both temperature stage and silicon-based formulation significantly affected antioxidant enzyme activities (Supplementary Table S1).**

These findings indicate that the observed enzyme activation reflected the general antioxidant response to heat stress, while the silicon-based formulation further enhanced this response. The consistently lower H_2O_2 and MDA levels in seedlings treated with the silicon-based formulation

support the interpretation that the silicon-based formulation improved the efficiency of the antioxidant defense system rather than acting as the sole trigger of enzyme activation.

Recent molecular studies indicate that silicon-mediated heat tolerance extends beyond antioxidant enzyme activation and involves the regulation of stress-responsive signaling pathways. Enhanced activities of SOD, CAT, and POD improve the capacity to scavenge reactive oxygen species (ROS), thereby protecting cellular components and maintaining redox homeostasis (Hasanuzzaman et al., 2018; Shomali et al., 2022). **In addition, silicon has been reported to promote proline accumulation through the regulation of pyrroline-5-carboxylate synthetase (P5CS) and other stress-responsive genes, contributing to osmotic adjustment, protein stabilization, and cellular protection under abiotic stress conditions** (Maghsoudi et al., 2018; Mao et al., 2025). **Although gene expression and signaling pathways were not investigated in the present study, the enhanced antioxidant enzyme activities, increased proline accumulation, and reduced H_2O_2 and MDA contents observed here are consistent with molecular mechanisms previously reported for silicon-mediated stress responses. The reduction in MDA content in plants treated with the silicon-based formulation suggests reduced lipid peroxidation and is consistent with improved membrane integrity under heat stress, although membrane stability was not directly measured in the present study.** Numerous studies have demonstrated that silicon reinforces cell wall structures and regulates membrane-associated processes, thereby limiting lipid peroxidation and maintaining membrane integrity under abiotic stress (Singh et al., 2023). The protective effect observed here aligns with previous findings on the role of silicon fertilizers in reducing lipid peroxidation under temperature and salinity stress.

In the present study, the coordinated reduction in H_2O_2 and MDA together with enhanced antioxidant enzyme activities and proline accumulation provides complementary biochemical evidence that the silicon-based formulation improved cellular redox homeostasis under heat stress. Although additional physiological indicators, such as electrolyte leakage or chlorophyll fluorescence, were not evaluated, the observed biochemical responses are consistent with enhanced cellular protection against heat-induced oxidative damage. Nevertheless, these findings should be interpreted as biochemical evidence of enhanced cellular protection rather than direct evidence of improved whole-plant heat tolerance.

Proline accumulation was enhanced under heat stress and further stimulated by the silicon-based formulation. The negative correlations observed between proline and H_2O_2, as well as between CAT activity and H_2O_2, indicate that both enzymatic and non-enzymatic antioxidant systems operate in a coordinated manner. Such coordinated integration of enzymatic and non-enzymatic defense mechanisms has been previously reported in plants exhibiting silicon-mediated stress tolerance (Rezende et al., 2017). **Since proline is a well-established osmoprotectant and antioxidant metabolite, its increased accumulation likely contributed to improved cellular protection under heat stress. Although the present study did not investigate the molecular regulation of proline metabolism, the observed biochemical response is consistent with previous reports describing silicon-associated increases in proline accumulation under abiotic stress. Therefore, our findings support an association between treatment with the silicon-based formulation and increased proline accumulation rather than providing direct evidence of silicon-mediated regulation of proline metabolism. Furthermore, the moderate negative correlation between proline and MDA ($r = -0.45$) suggests that increased proline accumulation was associated with reduced lipid peroxidation under heat stress. However, because this relationship was moderate rather than strong, the present findings do not support the interpretation that proline alone was the primary determinant of membrane stabilization. Instead, the reduction in MDA likely reflects the integrated action of enzymatic antioxidants together with non-enzymatic osmoprotectants, including proline, rather than the protective effect of a single metabolite** (De Ronde et al., 2000; Hnilickova et al., 2021).

Cultivar-dependent variation was evident. Although Bukhara-8 exhibited relatively higher basal antioxidant activity, Bukhara-102 showed stronger inducible responses under silicon-based formulation, suggesting differences in metabolic plasticity and redox regulation. **Genotypic variation in antioxidant capacity and heat tolerance has been widely documented in upland cotton, reflecting differences in ROS detoxification and stress adaptation among cultivars** (Khan et al., 2026; Kurbonov et al., 2026). **The present findings are consistent with these reports, indicating that cultivar-specific differences should be considered when evaluating biochemical responses to heat stress.**

The correlation analysis further supports the existence of a coordinated antioxidant defense network in cotton seedlings subjected to heat stress. The positive correlations among SOD, CAT, POD, and proline indicate that enzymatic and non-enzymatic antioxidant systems responded in a

coordinated manner to oxidative stress. In contrast, the negative correlations between H_2O_2 and antioxidant enzymes, as well as between H_2O_2 and proline, indicate that enhanced antioxidant capacity was associated with reduced oxidative stress. The positive association between H_2O_2 and MDA further confirms that increased ROS accumulation was accompanied by greater lipid peroxidation. These relationships complement the biochemical results presented in Tables 2 and 3 and support the conclusion that the silicon-based formulation strengthened antioxidant defense and reduced oxidative damage under heat stress.

Although the present study clearly demonstrates that the silicon-based formulation enhanced antioxidant defense and reduced oxidative damage under acute heat stress, the evaluation was limited to biochemical responses during the early seedling stage. Physiological indicators directly reflecting whole-plant heat tolerance, such as biomass accumulation, electrolyte leakage, survival rate, and photosynthetic performance, were not assessed. In addition, the study employed a single acute heat stress regime (45 °C for 6 h), which was selected to induce a controlled and reproducible oxidative stress response under laboratory conditions.

Although this approach is appropriate for investigating the immediate biochemical effects of heat stress, field conditions are typically more variable and prolonged. Furthermore, only two upland cotton cultivars were evaluated, and post-stress recovery was assessed only during the first 24 h following heat exposure. Therefore, the present findings should be interpreted within the scope of the experimental conditions and should not be generalized to all cotton genotypes or long-term field conditions.

An additional limitation of the present study is that the silicon-based formulation used in the experiment contained nitrogen in addition to silicon. Consequently, the individual physiological contributions of silicon and nitrogen could not be distinguished under the present experimental design. However, the primary objective of this study was to evaluate the biological effectiveness of the commercially available silicon-based formulation under controlled heat stress conditions rather than to isolate the effects of its individual components. Accordingly, the present findings should be interpreted within the context of evaluating the commercial formulation rather than the independent physiological effects of its individual constituents.

Future studies should include an equivalent nitrogen-only control treatment to distinguish the individual contributions of silicon and nitrogen. In addition, future research should integrate biochemical, physiological, and agronomic traits, evaluate a broader range of cotton cultivars, incorporate multiple post-stress recovery time points, and validate these findings under greenhouse and field conditions to provide a more comprehensive understanding of silicon-mediated heat tolerance in cotton.

Moreover, integrating gene expression analyses and molecular breeding approaches to identify silicon-responsive genes and molecular markers would further elucidate the mechanisms underlying silicon-mediated heat tolerance and facilitate the development of heat-resilient cotton cultivars.

In conclusion, short-term hyperthermia induced oxidative stress and growth suppression in cotton seedlings. Seed priming with the silicon-based formulation enhanced antioxidant defense capacity, reduced lipid peroxidation, and promoted proline accumulation, thereby strengthening biochemical protection against heat-induced oxidative stress. Within the scope of the present study, these findings demonstrate that the silicon-based formulation improved biochemical responses associated with protection against heat-induced oxidative damage during the early seedling stage. However, the present study did not evaluate long-term plant growth, productivity, or yield under heat stress, nor did it distinguish the individual physiological contributions of silicon and nitrogen contained in the commercial formulation. Therefore, further greenhouse and field studies, including an equivalent nitrogen-only control treatment, are required to determine whether these early biochemical responses translate into improved agronomic performance and heat tolerance throughout plant development.

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Table 1. Growth inhibition (%) of shoots and roots in two cotton cultivars under heat stress with and without the silicon-based formulation.

Cultivar	Shoots (%)		Roots (%)	
	Control	Aminosid-Silicon	Control	Aminosid-Silicon
Bukhara-8	74.2 ± 2.5 b	79.1 ± 0.5 a	93.4 ± 4.5 b	97.7 ± 4.2 a
Bukhara-102	68.9 ± 2.1 b	78.4 ± 0.3 a	94.1 ± 3.3 b	100.1 ± 3.1 a

Note: Values are presented as mean ± standard error (SE) of three biological replicates (n = 3). Growth inhibition (%) was calculated relative to the corresponding non-stressed control. Different lowercase letters within each cultivar indicate significant differences among treatment groups according to three-way ANOVA followed by Tukey's honestly significant difference (HSD) test ($P < 0.05$).

Table 2. Effect of the silicon-based formulation on antioxidant enzyme activities in cotton cultivars under heat stress and recovery conditions.

Cultivar	Control			Aminosid -Silicon		
	Before heat stress	After 6-hour exposure to 45 °C	Recovery (24 h at 30 °C)	Before heat stress	After 6-hour exposure to 45 °C	Recovery (24 h at 30 °C)
			SOD activity (U/mg protein)			
Bukhara-8	14.9±0.7 d	16.8±0.5 c	17.4±0.9 bc	15.9±0.7 c	17.1±0.5 bc	17.5±0.9 b
Bukhara-102	13.8±0.3 e	16.1±0.4 cd	16.9±0.6 c	16.2±0.3 bc	17.6±0.3 bc	19.4±0.6 a
			CAT activity (U/mg protein)			
Bukhara-8	132.0±4.8 c	136.1±2.5 bc	143.4±6.5 b	134.6±4.2 bc	138.1±2.9 b	145.6±4.4 a
Bukhara-102	125.4±4.1 d	124.3±4.5 d	139.3±4.5 b	131.3±4.0 c	139.1±3.4 b	145.7±4.0 a
			POD activity (U/mg protein)			
Bukhara-8	16.1±0.5 d	21.6±0.9 c	19.3±0.8 c	17.6±0.4 cd	24.1±0.9 b	27.8±0.8 b
Bukhara-102	15.4±0.5 d	24.0±0.7 b	22.0±0.6 bc	18.4±0.3 c	25.3±0.6 b	29.6±0.6 a

Note: Values are presented as mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters indicate significant differences among treatment combinations according to three-way ANOVA followed by Tukey's honestly significant difference (HSD) test ($P < 0.05$).

Table 3. Effect of the silicon-based formulation on hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and proline content in cotton cultivars under heat stress and recovery conditions.

Cultivar	Treatment	Before heat stress	45 °C (6 h)	Recovery (24 h at 30 °C)
		H_2O_2 (nmol g^{-1} FW)		
Bukhara-8	Control	147.7 ± 4 c	156.1 ± 5 a	118.1 ± 4 e
	Aminosid Silicon	141.6 ± 4 d	139.3 ± 5 d	119.2 ± 4 e
Bukhara-102	Control	149.5 ± 6 b	158.2 ± 7 a	128.3 ± 5 c
	Aminosid Silicon	129.4 ± 6 c	119.4 ± 7 d	117.1 ± 5 d
		MDA (nmol g^{-1} FW)		
Bukhara-8	Control	14.1 ± 0.3 c	17.6 ± 0.4 a	16.1 ± 0.3 b
	Aminosid Silicon	11.5 ± 0.4 e	14.3 ± 0.5 c	12.3 ± 0.5 d
Bukhara-102	Control	12.3 ± 0.4 c	14.7 ± 0.5 a	13.8 ± 0.5 b
	Aminosid Silicon	11.8 ± 0.3 d	13.9 ± 0.4 b	11.3 ± 0.3 d
		Proline (µmol g^{-1} FW)		
Bukhara-8	Control	0.51 ± 0.01 e	0.54 ± 0.01 d	0.65 ± 0.01 c
	Aminosid Silicon	0.64 ± 0.01 c	0.71 ± 0.01 b	0.75 ± 0.01 a
Bukhara-102	Control	0.67 ± 0.02 c	0.70 ± 0.01 c	0.83 ± 0.03 a

Aminosid Silicon	0.67 ± 0.02 c	0.74 ± 0.01 b	0.85 ± 0.03 a

Note: Values are presented as mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters indicate significant differences among cultivar × treatment × temperature combinations according to three-way ANOVA followed by Tukey's honestly significant difference (HSD) test ($P < 0.05$).

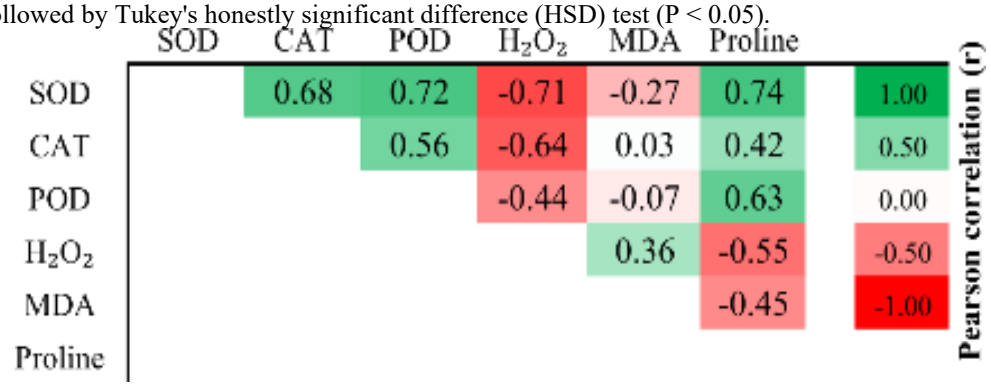

Figure 1. Pearson correlation heatmap illustrating the relationships among antioxidant enzyme activities (SOD, CAT, and POD), oxidative stress markers (H_2O_2 and MDA), and proline content in cotton seedlings subjected to heat stress with and without the silicon-based formulation. **Note:** Pearson correlation coefficients (r) are presented in each cell. Positive correlations are shown in green, negative correlations in orange, and values close to zero in white. Correlation analysis was performed using replicate-level data (n = 36) obtained from three biological replicates across two cotton cultivars, two treatment groups (control and the silicon-based formulation), and three temperature stages (before heat stress, heat stress, and recovery). The corresponding P-values and significance levels for all Pearson correlation coefficients are provided in Supplementary Table S2.