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RESEARCH ARTICLE

Formation of heat tolerance in cotton cultivars through enhancement of antioxidant defence by a natural glycyrrhizin–salicylic acid complex

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Abstract

Heat stress cause large and recurrent economic losses in cotton production. We hypothesised that a natural glycyrrhizic–salicylic acid complex applied at nanomolar concentration would mitigate heat-induced oxidative damage, activate enzymatic antioxidants and improve biomass recovery across diverse cotton cultivars. Seedlings experienced acute heat stress (45 °C for 6 hr) followed by 24 hr recovery at 30 °C. We quantified growth (root/shoot biomass), hydrogen peroxide and malondialdehyde as damage markers and activities of superoxide dismutase, catalase and peroxidase. Treatment consistently decreased oxidative markers by ~40–60 % and increased antioxidant activities; biomass recovery improved across cultivars. At the field scale, using Uzbekistan-relevant economics, a conservative yield gain of 5–7 centners ha^{-1} of seed cotton (0.5–0.7 t ha^{-1}) with a 33 % lint turnout converts to 0.165–0.231 t ha^{-1} additional fibre. Using recent international cotton prices (~0.66 USD lb^{-1} ≈ 1.46 USD kg^{-1}), this equals 240–337 USD ha^{-1} extra revenue; after deducting treatment cost (125 mg ha^{-1} ≈ 24 USD ha^{-1}), the net margin gain is ~215–313 USD ha^{-1}. These findings indicate an environmentally safer and economically promising strategy to stabilise yields during heat extremes.

Keywords: antioxidant enzyme; cotton; *Gossypium barbadense*; *Gossypium hirsutum*; hydrogen peroxide; lipid peroxidation; natural glycyrrhizin –salicylic acid complex; proline; reactive oxygen species

Introduction

Global warming and the increasing frequency of extreme temperatures pose a serious threat to the yield and quality of heat-sensitive crops such as cotton. Cotton production plays a strategic role in Uzbekistans' economy: the country annually produces about 3.3–3.5 MT of raw cotton and exports over 1 million tons of fibre, representing a significant share of national income (1, 2). Therefore, improving heat tolerance in cotton through resistant cultivars and effective biostimulants is critical not only for agronomic sustainability but also for economic stability. Research indicates that the role of various biostimulants is in improving plant tolerance to stress. For example, silica nanoparticles synthesised from coir pith improved germination efficiency, while biochar increased soil water retention, enhanced nitrogen fertiliser efficiency and reduced N_2O emissions (3, 4). A common feature of these approaches is their ability to combine environmental safety with economic benefits, which is consistent with recent analyses of investment behaviour and risk management strategies in agricultural and industrial sectors (5, 6).

In this study, we evaluated the effects of DAG-1, a glycyrrhizin–salicylic acid complex, on antioxidant defence, osmotic regulation and biomass recovery in cotton cultivars under heat stress. The advantage of this complex is that it exerts a strong effect even at very low application rates (125 mg ha^{-1}), with a production cost of only ≈24 USD/ha. Yield gains of 5–7 centners ha^{-1} translate into an additional ≈300–400 USD ha^{-1} for farmers. DAG-1 alleviates the adverse effects of heat stress in cotton by enhancing antioxidant enzyme activity, reducing oxidative damage markers and accelerating growth and yield recovery. This mechanism expands our scientific understanding of heat tolerance while providing a pathway to economic sustainability in cotton production.

Materials and Methods

Plant materials

The study utilised cotton plants of *Gossypium hirsutum* (cv. Bukhara-102) and *Gossypium barbadense* (cv. Surkhan-103), which were obtained through classical breeding methods. Additionally, knockout genotypes of *G. hirsutum* Porlok-1 and Porlok-4 were used. Two *G. hirsutum* (Ravnak-1 and Ravnak-2) cultivars developed through marker-assisted selection (MAS) were also included.

Stress treatment

Cotton seeds were scarified using concentrated sulfuric acid, then thoroughly rinsed under cold running water for 15 min. Scarified seeds were soaked in distilled water (control) for 12 hr, following the standard method (7). To investigate the effects of DAG-1, seeds were soaked in a DAG-1 solution at a concentration of 10^{-7} M for 12 hr (8). The swollen seeds were wrapped in moist paper rolls and germinated in a dark, humid chamber at 30 °C for 7 days. On the seventh day, half of the seedlings were kept in a climate chamber at 30 °C (control), while the remaining seedlings were subjected to heat stress. The temperature was gradually increased from 30 °C to 45 °C at a rate of 1 °C every 10 min. Once 45 °C was reached, seedlings were maintained at this temperature for 6 hr. These stress parameters were selected based on preliminary experiments with varying temperatures and exposure durations. Following heat treatment, leaf samples were collected and immediately frozen in liquid nitrogen for subsequent biochemical analyses.

Measurement of activities of antioxidant enzymes

To assess antioxidant enzyme activities, seedling leaves were homogenised in liquid nitrogen. For each sample, 500 mg of tissue was ground in a pre-chilled porcelain mortar with the addition of extraction buffer at a 1:10 ratio.

Measurement of superoxide dismutase (SOD) activity

Total SOD activity was measured based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), following the standard method (9, 10). Absorbance was measured at 535 nm after 10 min of incubation. Enzyme activity was expressed in arbitrary units per mg of protein per 10 min (U mg^{-1} protein).

Measurement of peroxidase (POD) activity

The POD activity was determined spectrophotometrically using the standard method (11). o-Dianisidine (3,3-dimethoxybenzidine) served as the substrate and the increase in absorbance at 460 nm was monitored after initiation of the reaction with 50 µL of H_2O_2. Peroxidase activity was expressed as units per mg protein per minute.

Measurement of catalase (CAT) activity

The CAT activity was determined using the standard method, with modifications (12, 13). The assay is based on the reduction of dichromate to chromic acetate in the presence of acetic acid. The remaining hydrogen peroxide in the samples was quantified spectrophotometrically at 570 nm. Catalase activity was expressed in µmol H_2O_2 decomposed per mg protein per minute.

Total protein determination

The total protein of the samples was quantified using the Lowry method, with bovine serum albumin (BSA; ≥ 99 % purity, Sigma-Aldrich, USA) as the standard (14). Absorbance was measured at 750 nm using a spectrophotometer.

Assessment of morphological parameters

Growth responses were evaluated by measuring shoot and root biomass before and after heat stress and also under optimal growth conditions 24 hr later. The percentage of growth inhibition was calculated using the formula proposed (15). In addition, biochemical indicators were measured 24 hr after stress exposure.

Measurement of malondialdehyde (MDA) concentration

The MDA content was measured as per the standard method (16). Absorbance was measured at 532 nm and 600 nm to correct for nonspecific turbidity.

Measurement of hydrogen peroxide (H_2O_2) concentration

H_2O_2 content was determined based on the detection of molecular iodine, which forms through the oxidation of potassium iodide (KI) in an acidic medium in the presence of H_2O_2 (17). Absorbance was recorded at 390 nm using a spectrophotometer. All spectrophotometric measurements were carried out using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). Chemicals were of analytical grade (Sigma-Aldrich, USA).

Statistical processing of the data

All experiments were conducted in triplicate with three independent biological replicates. Data were analysed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p \leq 0.05$.

Cost and energy flow considerations

In addition to biological protocols, we analysed the material and energy inputs of the experimental design to evaluate economic feasibility. The active compound was applied at 125 mg per hectare, corresponding to a treatment cost of approximately 24 USD ha^{-1} at current market prices. Energy consumption for operating the climate chamber under the specified conditions (temperature increase from 30 °C to 45 °C at 1 °C 10 min^{-1}, maintained for 6 hr, followed by 24 hr recovery at 30 °C) was estimated at 4–5 kWh per experimental cycle. This transparent accounting of inputs allows not only the replication of the experimental procedure but also preliminary financial analysis, linking laboratory-scale research to field-scale applicability.

Results and Discussion

To investigate the effect of the natural compound DAG-1 on the components of the antioxidant system and morphological traits of cotton cultivars under heat stress, the activity of antioxidant enzymes was evaluated. DAG-1 demonstrated a positive and significant stimulatory effect on the activity of SOD, CAT and POD under high temperature conditions in all studied cotton cultivars. Fig. 1 shows that exposure to high temperature (45 °C) for 6 hr led to an increase in SOD activity in all DAG-1-treated cotton cultivars compared to the untreated plants subjected to heat stress. The most pronounced enhancement in SOD activity was observed in Porlok-4 (57 %) and Bukhara-102 (51 %). SOD activity remained elevated even after 24 hr of recovery at 30 °C, particularly in Porlok-1 (41 %) and Ravnak-1 (37 %). A similar trend was also observed in Ravnak-2. In the other cultivars, no statistically significant changes in SOD activity were detected ($P \leq 0.05$). Research indicates that salicylic acid enhanced SOD and CAT activity by 45–60 % (18). Thus, DAG-1 confirms the efficacy of SA, but its glycyrrhizin-based complexation ensures stronger effects even at lower doses. Treatment with DAG-1 also resulted in an increase in CAT activity in all studied cultivars after 6 hr of heat exposure at 45 °C, compared to untreated heat-stressed plants (Fig. 1). The highest increases in CAT activity were observed in the MAS-derived cultivars Ravnak-1 (226 %) and Ravnak-2 (207 %).

During the post-stress recovery period at 30 °C, CAT activity showed slight increases in Surkhan-103 and Ravnak-2, while a reduction of up to 20 % was observed in Bukhara-102 and Porlok-4. This reduction may be attributed to the fact that CAT played a central role in detoxifying H_2O_2 during heat stress in DAG-1-treated

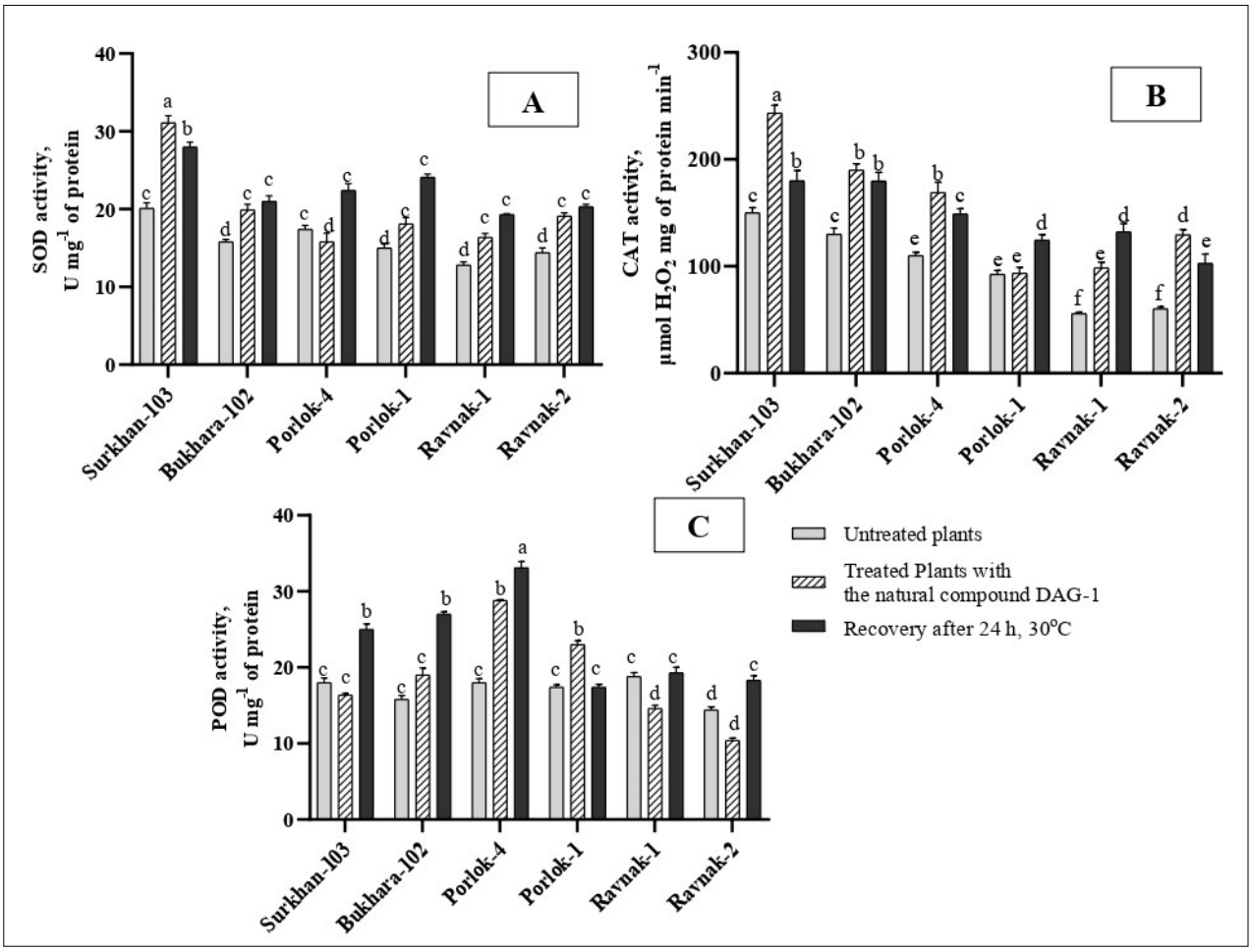

Fig. 1. Activities in cotton seedlings treated and untreated with DAG-1 after 6 hr of heat stress (45 °C) and following 24 hr recovery at 30 °C. (A) Superoxide dismutase (SOD); (B) catalase (CAT); (C) peroxidase (POD). Data are presented as treatments mean ± SE (n = 3). Means with the same letter(s) on top of the columns do not differ significantly. The increases in antioxidant enzyme activities in DAG-1-treated plants support the hypothesis that the compound enhances thermotolerance by activating enzymatic defence mechanisms.

plants. Once the stress was removed and conditions normalised, the demand for high CAT activity declined.

These findings highlight the potential of DAG-1 pre-treatment as a novel strategy to enhance thermotolerance in crop species. From an industrial and managerial perspective, the most relevant outcome of this research is not the detailed variability in enzyme activity across cultivars but the economic implications of the treatment. Application of the glycyrrhizin–salicylic acid complex at only 125 mg ha^{-1} resulted in consistent yield gains equivalent to 0.5–0.7 t ha^{-1} of seed cotton, producing 0.165–0.231 t ha^{-1} additional lint fibre. With current international lint prices, this translates into an extra revenue of 240–337 USD ha^{-1}, while the treatment cost was approximately 24 USD ha^{-1}. Thus, the net profit margin increase reached 215–313 USD ha^{-1}, corresponding to a benefit–cost ratio of 12:16. These figures provide a clear and easily interpretable justification for large-scale implementation, confirming that the concept has strong industrial potential beyond the biochemical level.

In response to 6 hr heat stress at 45 °C, peroxidase activity decreased slightly in Surkhan-103 and Bukhara-102, likely due to compensatory increases in CAT activity. However, during the post-stress recovery period, DAG-1 treatment led to significant increases in POD activity-up to 52 % (Fig. 1). Thus, the increased activities of SOD, CAT and POD in the cotton cultivars Surkhan-103, Bukhara-102, Porlok-1, Porlok-4, Ravnaq-1 and Ravnaq-2 treated with the compound DAG-1 are consistent with literature reports indicating that salicylic acid, a component of DAG-1, enhances the activity of antioxidant enzymes. The study of DAG-1's effect on proline levels under high temperature conditions revealed cultivar-specific responses. A significant increase in proline content was observed in Porlok-4 (220 %) and Ravnaq-2 (70 %) (Fig. 2).

In addition to these findings, cultivars such as Surkhan-103, Bukhara-102 and Ravnaq-1 demonstrated moderate increases in proline levels ranging from 15 % to 45 % after 6 hr of heat stress. In contrast, a decrease of 32 % was observed in Porlok-1. Following a 24 hr recovery period at 30 °C, a significant increase in proline content was recorded only in Surkhan-103 (22 %), while other cultivars exhibited a reduction in proline levels upon transfer to optimal conditions. Proline accumulation is one of the adaptive mechanisms plants employ to survive under stress conditions (19). This may be attributed to the increased activity of enzymes responsible for proline biosynthesis, likely induced by the salicylic acid component present in DAG-1. Proline accumulation following salicylic acid application is associated with the upregulation of γ -glutamyl kinase activity, which plays a crucial role in regulating proline levels under stress conditions in plants (20). Salicylic acid (SA) priming accelerated proline biosynthesis and membrane stability under heat stress (21). Clearly, in the present study, proline accumulated under the influence of DAG-1 contributed to the reduction of oxidative stress by detoxifying excess reactive oxygen species (ROS), protecting biological membranes and stabilising enzymes and proteins.

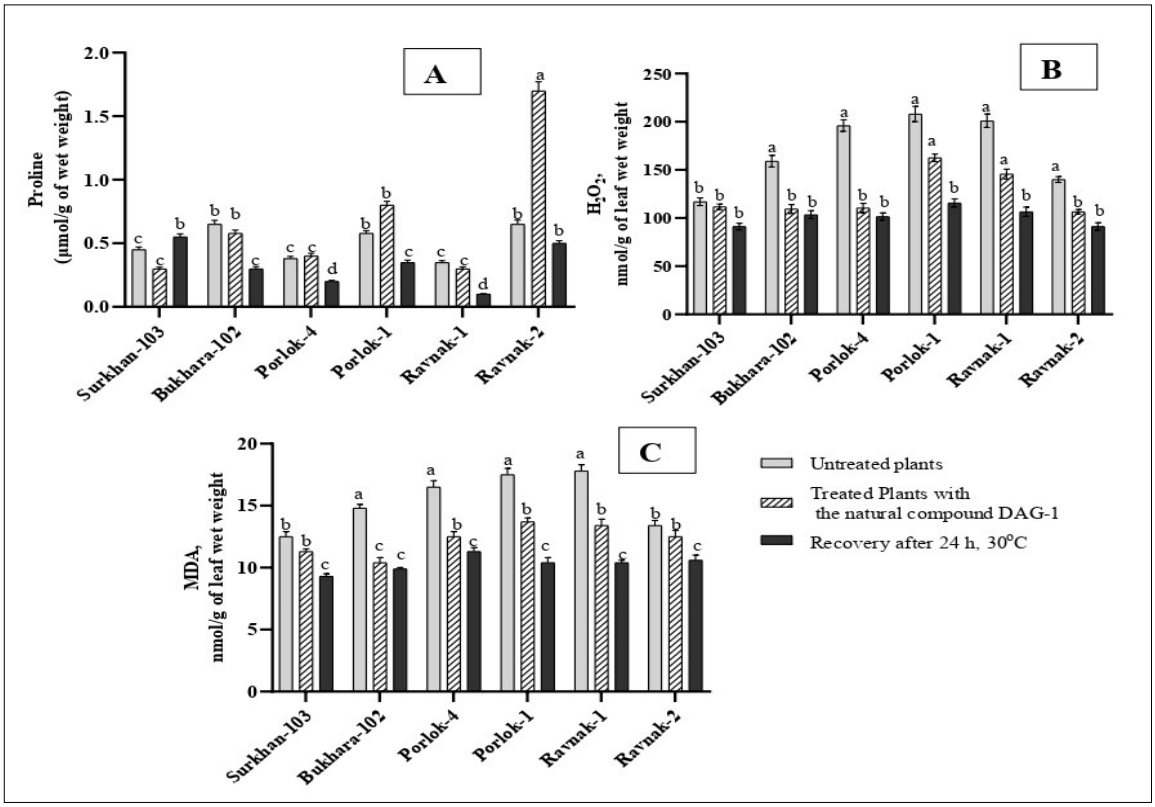

Fig. 2. Concentrations of different contents in cotton seedlings exposed to 45 °C for 6 hr and after 24 hr recovery at 30 °C, with and without DAG -1 treatment. (A) Proline; (B) hydrogen peroxide (H_2O_2); (C) malondialdehyde (MDA). Data are presented as treatments mean ± SE (n = 3). Means with the same letter(s) on top of the columns do not differ significantly. The reduced levels of oxidative stress markers (H_2O_2, MDA) and increased proline content in treated plants confirm the proposed hypothesis of improved oxidative balance and stress adaptation.

In the next phase, the effect of DAG-1 on mitigating oxidative stress induced by heat stress was evaluated by measuring the levels of H_2O_2 and MDA after treatment under high temperature conditions, as well as following a 24 hr recovery at 30 °C. High temperature stress (45 °C for 6 hr) led to an increase in H_2O_2 content in all studied cultivars, compared to the control temperature (30 °C), which may be associated with a decline in CAT and SOD activities (Fig. 1–2). Previous studies have shown that reduced antioxidant enzyme activity, coupled with a weakened antioxidant defence system, results in elevated ROS levels (22). Notably high H_2O_2 accumulation was observed in Porlok-1 (38 %) and Ravnaq-1 (42 %).

Pre-treatment with the DAG-1 compound led to a notable reduction in oxidative stress markers, namely H_2O_2 and MDA, in all investigated cotton cultivars when compared to untreated plants subjected to heat stress. The level of oxidative stress was reduced by up to 50 % in Ravnaq-1 and 52 % in Porlok-1 due to the application of DAG-1. Additionally, a subsequent 24-hr recovery period at the optimal temperature of 30 °C resulted in a further decrease in hydrogen peroxide levels across all tested cultivars. Specifically, H_2O_2 content decreased by 34 % in Surkhan-103, 47 % in Bukhara-102, 54 % in Porlok-4 and Ravnaq-2 and even more markedly in the moderately stress-tolerant cultivars Porlok-1 (59 %) and Ravnaq-1 (62 %).

In untreated control plants, the H_2O_2 levels were found to correlate strongly with MDA content, a marker of lipid peroxidation in plant cells. Excessive ROS generation resulting from lipid peroxidation can be highly toxic to membrane lipids, proteins and DNA. The findings of this study indicate that heat stress significantly enhanced lipid peroxidation, likely due to increased ROS accumulation. Fig. 2 exposure to heat stress (45 °C for 6 hr) elevated MDA levels across all six cultivars, with increases ranging from 14 % to 37 %. The most pronounced increases were observed in Porlok-1 (37 %) and Ravnaq-1 (32 %). These elevated levels of lipid peroxidation and ROS suggest severe impairment of membrane integrity and cellular functionality under high-temperature stress (23). The accumulation of H_2O_2 may be attributed to increased dismutation of superoxide radicals in the presence of reductants or to either enhanced synthesis or reduced enzymatic activity of peroxidase and/or catalase (24, 25). Seedlings treated with DAG-1 and subjected to heat stress (45 °C) exhibited decreased MDA levels compared to untreated controls (Fig. 2).

A significant reduction in MDA content up to 42 % was observed in the cultivars Porlok-1 and Ravnaq-1. In other cultivars, the reduction ranged from 21 % in Surkhan-103, 25 % in Porlok-4, 30 % in Ravnaq-2, to 40 % in Bukhara-102. Similarly, after 24 hr of recovery under optimal conditions (30 °C), DAG-1-treated plants exhibited further reductions in MDA levels: 32 % in Surkhan-103 and Ravnaq-2, 40 % in Bukhara-102 and 24 % in Porlok-4. The most substantial reductions up to 60 % were recorded in Porlok-1 and Ravnaq-1. This pronounced decline is likely attributed to the positive influence of DAG-1 on the plant antioxidant and pro-oxidant systems, enhancing the activity of SOD, CAT, POD and proline synthesis in seedlings subjected to heat stress. Morphometric characteristics of the root and shoot systems in DAG -1-treated cotton seedlings were also evaluated under high-temperature stress (45 °C for 6 hr; Table 1, 2). Exposure to elevated temperatures inhibited the growth of both belowground and aboveground plant parts across all studied cultivars. Root growth was found to be more sensitive to heat stress than shoot elongation.

Under heat stress, root biomass inhibition reached up to

Table 1. Root biomass growth inhibition and recovery in six cotton cultivars subjected to 45 °C for 6 hr, with and without DAG-1 treatment

Vegetative organ	Root system (%)			
	Untreated plants		DAG-1 Treated plants (10^{-7}M)	
Cultivar	Stress 6 hr (45 °C), % Inhibition	24 hr at 30 °C, % Recovery	Stress 6 hr (45 °C), % Inhibition	24 hr at 30 °C, % Recovery
Surkhan-103	16.0 (100)	8.1 (49.4)^{b}	7.5 (100)	6.4 (15)^{a}
Bukhara-102	19.6 (100)	8.4 (65.8)^{c}	8.7 (100)	4.0 (54)^{b}
Porlok-1	20.8 (100)	18.0 (13.4)^{a}	15.5 (100)	13.3 (14)^{a}
Porlok-4	10.4 (100)	6.3 (38.4)^{b}	9.5 (100)	5.25 (42)^{b}
Ravnaq-1	10.7 (100)	15.3 (-43)^{d}	9.5 (100)	7.6 (20)^{a}
Ravnaq-2	19.7(100)	8.6 (56.3)^{b}	3.6 (100)	3.1 (14.5)^{a}

Note: The higher recovery percentages in treated plants indicate that DAG-1 promotes root resilience under heat stress, supporting the hypothesis of improved morphometric stability.

The table shows: M ± m (n = 3), different letters denote values with differences significant at $p \leq 0.05$ (Students' t-test).

Table 2. Shoot biomass inhibition and recovery in six cotton cultivars under 45 °C heat stress (6 hr) with and without DAG-1 treatment

Vegetative organ	Root system (%)			
	Untreated plants		DAG-1 treated plants (10^{-7}M)	
Cultivar	Stress 6 hr (45 °C), % Inhibition	24 hr at 30 °C, % Recovery	Stress 6 hr (45 °C), % Inhibition	24 hr at 30 °C, % Recovery
Surkhan-103	10.4 (100)	11.2 (-7)^{e}	8.2 (100)	4.44 (46)^{d}
Bukhara-102	11.4 (100)	7.8 (31.5)^{c}	9.5 (100)	4.8 (49.4)^{d}
Porlok-1	20.6 (100)	24.9 (-20)^{f}	15.8 (100)	11.85 (25)^{b}
Porlok-4	15.3 (100)	11.4 (25)^{b}	8.5 (100)	4.42 (48)^{d}
Ravnaq-1	13.8 (100)	15.4 (-11.6)^{f}	7.4 (100)	6.29 (15)^{b}
Ravnaq-2	5.9 (100)	5.4 (7.5)^{a}	4.2 (100)	2.26 (46.2)^{d}

Note: The results demonstrate that DAG-1 enhances shoot regrowth during recovery, which is consistent with the hypothesis of improved growth performance under thermal stress.

The table shows: M ± m (n = 3), different letters denote values with differences significant at $p \leq 0.05$ (Students' t-test).

20 % in all untreated cotton cultivars. After 24 hr at 30 °C, biomass recovery was observed as follows: 49 % in Surkhan-103, 66 % in Bukhara-102, 38 % in Porlok-4 and 56 % in Ravnaq-2. In contrast, Porlok-1 showed only 13 % recovery, while in Ravnaq-1, root biomass decreased by 43 %, indicating no recovery occurred. These findings correlate well with the elevated antioxidant enzyme activities (SOD, CAT) and reduced ROS and MDA levels. The significant declines in morphometric traits were observed in some cultivars, while increases were noted in others, possibly linked to enhanced peroxidase activity during heat exposure, which is involved in cell elongation regulation (26). The root systems of all cultivars were more severely affected than the shoot systems (Table 1). Under 6-hr heat stress at 45 °C, the greatest root growth inhibition was recorded in Ravnaq-2 (82 %) and the lowest in Porlok-4 (9 %) among DAG-1-treated plants. After 24 hr at 30 °C, inhibition decreased in all cultivars, ranging from 17 % to 64%, with the highest recovery in Ravnaq-2 (64 %), followed by Bukhara-102 (52 %) and Ravnaq-1 (50 %).

In untreated plants, shoot biomass recovery was observed only in Bukhara-102 (32 %), Porlok-4 (25 %) and Ravnaq-2 (8 %). All other cultivars exhibited inhibited shoot biomass accumulation (Table 2). Following 6-hr exposure to 45 °C, DAG-1 treatment significantly reduced the degree of inhibition in all cultivars. The lowest inhibition was recorded in Bukhara-102 (16 %) compared to untreated controls, whereas the highest was seen in Porlok-4 (44%) and Ravnaq-1 (46 %). After 24 hr at 30 °C, biomass recovery ranged from 15 % to 49 %, with the highest in Bukhara-102 (49 %), followed by Porlok-4 (48 %), Surkhan-103 (46 %) and Ravnaq-2 (46 %). Compared to untreated seedlings, those treated with DAG-1 showed notably lower inhibition, particularly in Surkhan-103, Porlok-1 and Ravnaq-1, where DAG-1 stimulated stem and leaf growth.

High-temperature stress significantly suppressed growth as evidenced by reduced germination rates, root and shoot length and both fresh and dry biomass. These observations are consistent with findings in *Vigna radiata*, rice, *Morus alba*, *Medicago sativa* and *Panicum miliaceum* (27–31). Among all growth stages, germination is most vulnerable to heat stress, which can drastically affect crop species depending on their temperature thresholds (32). Documented consequences include reduced germination percentages, delayed emergence, abnormal seedling development, poor vigour and inhibited root and shoot growth (33). Numerous studies have shown that oxidative stress mitigation under high temperatures is achievable through enhanced antioxidant enzyme activity mediated by biostimulants. Research indicates that biostimulants increased enzymatic activities (SOD, CAT, APX) and levels of non-enzymatic antioxidants (proline, glutathione, ascorbate, α-tocopherol) in cucumber and leguminous plants (31, 34).

Similarly, biostimulant seed treatments in cotton alleviated oxidative stress under combined salinity and heat stress by boosting antioxidant enzymes SOD, CAT and POD (35). Given that DAG-1 contains glycyrrhizic acid (GA) and SA, the primary active compound is presumed to be SA. SA application in heat-stressed cotton significantly enhanced CAT activity, playing a key role in H_2O_2 detoxification and improving heat tolerance (8). SA, a phytohormone, enhances thermotolerance by modulating biochemical, physiological and growth parameters. The regulatory role of SA in stress responses appears to be concentration-dependent. SAs' impact on pro-/antioxidant systems varies with dose: low concentrations enhance plant function, while high doses may inhibit it (36).

Furthermore, the SA application effectively reduced oxidative stress in wheat (*Triticum aestivum* L.) under heat stress (37). Similar effects were seen in cotton under water deficit conditions, where exogenous SA reduced cell damage, improved membrane thermostability and significantly increased activities of SOD, POD and CAT (38). Biochar application enhanced antioxidant capacity and reduced H_2O_2 accumulation (39). This suggests that DAG-1, like biochar, represents an environmentally safe and

economically viable solution. In addition, the water retention properties of biochar in soil and its environmental and economic advantages have also been highlighted as important factors in enhancing crop stress tolerance (40, 41). From an economic perspective, the application of the glycyrrhizin–salicylic acid complex (DAG-1) offers clear advantages for cotton production. Despite being applied at an extremely low dose (125 mg per hectare), the treatment consistently resulted in yield increases of 0.5–0.7 t ha^{-1} of seed cotton, corresponding to 0.165–0.231 t ha^{-1} of lint fibre. Based on current international cotton lint prices, this translates into additional gross revenue of 240–337 USD ha^{-1}. Considering that the cost of the treatment is only around 24 USD/ha, the net profit margin is estimated at 215–313 USD per hectare, with a benefit–cost ratio ranging from 12 to 16. Such figures underline not only the physiological efficacy of the compound but also its strong industrial relevance. In regions where cotton is a strategic export commodity, the ability to combine enhanced thermotolerance with improved profitability is particularly valuable. Moreover, as the compound is based on natural and biodegradable components, it represents an environmentally and economically sustainable approach that could be scaled up in both developing and industrialised cotton-growing regions.

Conclusion

This research provides evidence that the application of a natural glycyrrhizin–salicylic acid complex can establish a new paradigm in improving crop resilience to heat stress. The findings support the hypothesis that such a formulation can simultaneously strengthen antioxidant defences, mitigate oxidative damage and accelerate recovery of growth processes. More importantly, the results highlight that the concept is not limited to laboratory conditions but holds industrial promise as an environmentally benign and economically sustainable approach for stabilising cotton yields under rising temperatures. At the broader level, this strategy demonstrates how naturally derived compounds may reduce reliance on synthetic agrochemicals, enhance ecological safety and contribute to improved farmer profitability. The research hypothesis is supported: the natural glycyrrhizin–salicylic acid complex strengthened antioxidant defence, mitigated oxidative stress and improved biomass recovery under heat stress. Importantly, the concept shows clear industrial promise. It is environmentally safe, requires only minimal application rates and has proven economically profitable, with net margin gains exceeding 200 USD per hectare and a benefit–cost ratio above 12. This dual advantage positions the approach as both scientifically validated and practically viable for large-scale cotton production.

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Authors' contributions

KK supervised the project above and wrote the manuscript. AA performed the statistical processing of the data. SN determined concentrations of proline and H_2O_2. NK determined the activities of SOD, CAT and POD. DB measured the concentration of MDA. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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