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HEAT STRESS EFFECTS ON PHYSIOLOGICAL AND BIOCHEMICAL FEATURES OF COTTON UNDER HYPERTHERMIA

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SUMMARY

Oxidative stress caused by the accumulation of reactive oxygen species (ROS) produces harmful toxic effects on plant cells and exposes the cotton crop to high temperatures, which are the major limiting factors causing yield losses. Every cotton genotype under heat stress, the pro/antioxidant being a key system among the biochemical factors contributing to plant protection, entailed studies in two cotton cultivars, Surkhan-103 (*G. barbadense* L.) and Bukhara-102 (*G. hirsutum* L.) in the laboratory and field conditions. The antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), peroxidase (PO), malondialdehyde (MDA), and proline, underwent evaluation under prolonged (6-hour) exposure to high temperature (45 °C). The results revealed cultivars Surkhan-103 and Bukhara-102 are classifiable as resistant to heat stress by their biochemical parameters; an increase in the concentrations of hydrogen peroxide and MDA with activities of antioxidant enzymes could become the key markers of plant tolerance and intolerance to heat stress. Under high-temperature conditions, the fine-fiber cultivars Surkhan-104 and Surkhan-103, the medium-fiber cultivars Bukhara-102, S-6577, and S-6585, and the advanced lines L-688, L-214, and L-403 exhibited considerable stability. These heat-tolerant genotypes achieved recommendation for use in future breeding programs aimed at improving stress resilience.

Keywords: *G. barbadense* and *G. hirsutum*, hyperthermia, malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase, reactive oxygen species (ROS), proline

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Key findings: The study comprising the hyperthermia effect on cotton (*G. barbadense* and *G. hirsutum*) cultivars revealed significant insights into plant responses to heat stress. Both cultivars demonstrated resilience by maintaining higher activities of key antioxidant enzymes under prolonged exposure to high temperatures.

INTRODUCTION

High-temperature stress is a leading abiotic stress factor in the cultivation of heat-loving crops like cotton (*G. barbadense* and *G. hirsutum*), determining their productivity. Heat stress affects all aspects of the physiology and biochemistry of plants, causing excessive accumulation of the reactive oxygen species (ROS) toxic toward plant cells. The species include free radicals, superoxide (O_2), hydroxyl (OH), peroxyl (ROO), hydrogen peroxide (H_2O_2), singlet oxygen ($1O_2$), and ozone (O_3) (Khan *et al.*, 2017).

Hydrogen peroxide is a rather stable reactive oxygen species and a dismutation of superoxide. In low concentrations, hydrogen peroxide transmits signals causing counteraction to biotic and abiotic stress; meanwhile, in high concentrations, it also causes the programmed cells' death (Quan *et al.*, 2008). In crop plants, the highest and lowest temperatures cause enhancement in the membrane lipid peroxidation induced by excessive ROS accumulation. This eventually causes an increase in malondialdehyde (MDA) concentration that plays a crucial role in cell membrane damage by free radicals in crop plants (Wahid *et al.*, 2007).

Superoxide dismutase (SOD), catalase (CAT), and peroxidase (PO) have key roles in the neutralization of the hydrogen peroxide toxicity, and plant protection from free radicals are three major antioxidant enzymes (Hasanuzzaman *et al.*, 2020). Among all the antioxidant enzymes, SOD and CAT are the most efficient ones; their combined effect neutralizes potentially dangerous superoxide anion radicals, catalyzing decomposition of hydrogen peroxide into water and molecular hydrogen and preventing cell damage (Abd-El-Haleem *et al.*, 2010). Moreover, PO catalyzes the recovery of hydrogen peroxide using electrons with various molecules as donors; the thermal stability of the PO molecules is rather high.

Proline typically plays a significant role in the metabolism of crop plants subjected to various stress factors, particularly high temperatures. It serves as a source of carbon and nitrogen and as a membrane stabilizer (Radyukina *et al.*, 2008). In cotton, if the temperature is higher than optimal (32 °C), the balance of the pro/antioxidant system shifts toward the latter; however, when the temperature reaches a critical point, membrane permeability increases, disrupting the ultrastructure of the cytoplasm and mitochondria, affecting the enzymes' activity (Mohamed and Abdel-Hamid, 2013). Consequently, the cotton pollen loses viability, leading to poor pollen germination and tube formation (Snider *et al.*, 2009).

For this reason, a study of cotton cultivars commenced based on various activities of the major components of the antioxidant system, such as SOD, CAT, PO, and ROS, as well as proline and MDA under thermal effect. The research will provide an opportunity to define essential methodological approaches to the integrated assessment of a breeding material's adaptive capacity to hyperthermia. The main objective of this study is to investigate the response of cotton to heat stress and its adaptation mechanisms, particularly by analyzing the effects of such stress on the plant's physiological and biochemical characteristics.

MATERIALS AND METHODS

Plant material

The following study used two cotton cultivars generated through conventional breeding, Surkhan-103 (*G. barbadense* L.) and Bukhara-102 (*G. hirsutum* L.). The seeds came from the

Cotton Breeding, Seed Production, and Agrotechnology Research Institute, Tashkent, Uzbekistan. An experiment in the Phytotron greenhouse also ensued, using seven fine-fiber cotton cultivars, including Surkhan-103, Surkhan-104, Surkhan-105, Surkhan-106, Surkhan-111, Surkhan-14, and Surkhan-16. In the phytotron, other materials comprised seven medium-fiber cotton cultivars, such as Bukhara-102, Namangan-77, S-6577, S-6585, C-6524, Sultan, and Jarkurgan, and new advanced lines—L-403, L-604, and L-214.

Seedling production

Seeds of the cotton cultivars under study sustained denuding in concentrated sulfuric acid, washing with running water for 15 min, and remained in distilled water for 12 h. The swollen seeds incurred wrapping in paper bags before being germinated in a dark, wet chamber for seven days at 30 °C.

Processing in the climatic chamber

After the seventh day of germination, 50% of the sprouts went into the climatic chamber (FLI-250, USA) at 30 °C as the control; to simulate heat stress, the increasing temperature reached from 30 °C to 45 °C, rising by 1 °C every 10 min. Upon reaching the desired temperature, the plants entailed exposure to 45 °C for six hours (Kolupaev *et al*., 2023). After exposure to the heat stress, the leaf samples succeeded in being collected to be frozen in liquid nitrogen and subsequently analyzed. The second stage of the study included the determination of activities of SOD, catalase, and PO, as well as concentrations of hydrogen peroxide, proline, and MDA in 24 h after elimination of heat stress at 30 °C. The seedlings remained at 45 °C for 6 h. The inhibition of seedlings and root growth of the seedlings underwent determination according to Kolupaev *et al.* (2023).

Activities of antioxidant enzymes

The activities of various antioxidant enzymes attained determination using the seed leaves of cotton sprouts homogenized in liquid nitrogen. In obtaining the extracts, a 500-mg sample of tissue received grinding in a cold mortar with the addition of appropriate extraction buffer in a 1:10 ratio.

Total SOD activity

The total SOD activity determination continued by measuring its capacity to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) chloride in accordance with the method used by Giannopolitis and Ries (1977), with some modifications (Polesskaya *et al*., 2004). The absorption, as measured spectrophotometrically, was at 535 nm (UV-1800, SHIMADZU, USA).

PO activity

The detection of the PO activity used hydrogen peroxide as a substrate and o-dianisidine as a reducing agent, using the methodology described by Christensen *et al*. (1998). The deviations in absorbance during the reaction reached determination at 460 nm (UV-1800, SHIMADZU, USA). Expressing the activity of POD was in U mg^{-1} of protein/min. The use of the Lowry method measured the protein (Lowry *et al*., 1951).

CAT activity

The CAT activity assessment occurred by the method where the reduction of dichromate in acetic acid to chromic acetate ensued. The process included heating in the presence of undecomposed hydrogen peroxide with the formation of perchromic acid as an unstable intermediate (Sinha, 1972), with some modifications (Hadwan, 2016). The absorption measurement proceeded spectrophotometrically at 570 nm (UV-1800, SHIMADZU, USA).

Hydrogen peroxide

The hydrogen peroxide determination was successful in accordance with the method presented by Junglee *et al*. (2014) and based on the potassium iodide (PI) oxidation by

hydrogen peroxide in the acidic medium. Measuring the absorption spectrophotometrically was at 390 nm (UV-1800, SHIMADZU, USA).

MDA concentration

The MDA concentration measurement was in accordance with the methodology of Gür *et al*. (2010) based on the formation of malondialdehyde product in reaction with thiobarbituric acid upon heating. The absorption estimation proceeded spectrophotometrically at 532 nm (UV-1800, SHIMADZU, USA).

Proline concentration

The proline concentration approximation was according to Shikhaleeva *et al*. (2014) and calculated using a calibration curve plotted using chemically pure proline (Himedia, India). The absorption succeeded in measuring spectrophotometrically at 520 nm (UV-1800, SHIMADZU, USA).

Statistical analysis

The experiments transpired in 2022, with three repetitions. Data assessment used analysis of variance (ANOVA) by SPSS statistics (ver. 17.0), with treatment mean presented as ±SE (n = 3). The least significant difference ($LSD_{0.05}$) sustained calculation for the significant means' difference. Bars showing the same letter were not significantly different by $LSD_{0.05}$.

RESULTS AND DISCUSSION

The results revealed a reduction in the activities of antioxidant enzymes (SOD and CAT), which could explain the thermal stress observed after prolonged (6 hours) exposure to high temperatures (45 °C). In plant adaptation to oxidative stress, the antioxidant enzyme activities can increase and decrease by the extent of temperature increase and its duration effect. High temperatures within a short period stimulate the biochemical processes and activate antioxidant enzymes, resulting in a reduction of oxidative stress. However, the prolonged temperature effects cause denaturation of their activities. For example, the short (1 hour) effect of 42 °C in 28 out of 37 genotypes caused the CAT activity to increase up to 333.3% on the seventh day of *Vigna aconitifolia,* associating the CAT activity increase with the thermal resistance (Harsh *et al*., 2016).

A significant increase in the activities of the antioxidant enzymes (PO, CAT, ascorbate peroxidase [APO], SOD) was evident after a 3-hour exposure at 42 °C, while after a 6-hour exposure, the activities of these four antioxidant enzymes decreased (Song *et al*., 2014). Likewise, Gür *et al.* (2010) reported the 2-hour effect of 45 °C in the heat-tolerant cultivar Stoneville-453 (*G. hirsutum* L) caused an increase in the activities of CAT and APO. Considerably, the rise in the activities plays a protective role in the short-term effect of hyperthermia and not causing significant oxidative stress. After a 2-hour effect, the MDA concentration was nonsignificant compared with the control plants. The studies on poplar demonstrated that the MDA concentrations had no substantial change even after three hours, probably associated with the escalation in the activities of antioxidant enzymes (SOD, CAT, and APO) (Song *et al*., 2014). The authors believe the accumulation of MDA and H_2O_2 appeared only after a 6-hour effect of high temperature with a reduction in the activities of antioxidant enzymes.

The reductions in activities of antioxidant enzymes seemed to parallel the decrease in SOD and CAT movements, suggesting that they may cause the reduction in H_2O_2. Contrary to SOD and CAT activities, the PO activity in the prolonged (6-hour) effect of high temperature showed to increase in both cotton species (Figure 1). Previous studies demonstrated the activities of novel isoforms of PO proved to rise after a 6-hour effect of 40 °C in heat-resistant cotton genotypes (Giza-85 and Giza-92), contrary to the heat-susceptible ones (Giza-80 and Giza-90) (Mohamed and Abdel-Hamid, 2013). Based on the findings, the low accumulation of malondialdehyde as the main indicator of lipid peroxidation of

membrane lipids was due to the PO activity in the cultivars Surkhan-103 and Bukhara-102. In previous studies, the PO activity in *Macrotyloma uniflorum* with the high temperature (43 °C–45 °C) effect occurred parallel with the reduction in the CAT activity (Naji and Devaraj, 2011). The CAT became the main enzyme participating in the metabolism of H_2O_2 produced by SOD, and its inhibition probably promotes the increase in the H_2O_2 concentration in the cultivar Bukhara-102 (Figure 1). In the cultivar Surkhan-103, the increased concentration of H_2O_2 under heat stress seemed probably correlated with the higher concentration of SOD.

Additionally, antioxidant enzyme accumulation, such as proline, manifested under the stress effect to neutralize the ROC. Past investigations demonstrated how the proline concentration in rice increased with short-term exposure to high temperature and reduced after long-term exposure. The said study reported that exposure of three rice cultivars to various temperatures (25 °C, 25 °C, and 40 °C) within 10 days for five hours caused an upsurge in proline concentration at 25 °C and 35 °C, while the reduction took place at 40 °C (Sánchez-Reinoso *et al*., 2014). The presented results revealed a decline in proline concentration in cotton cultivars Surkhan-103 and Bukhara-103 by 29% and 40%, respectively, under a 6-hour exposure to 45 °C, while in 24 hours at 30 °C, restoration of the concentration took place (Figure 2). For heat tolerance, similar results were notable in two cotton cultivars (Giza-85 and Giza-92), contrary to two other genotypes—Giza-80 and Giza-90 (Mohamed and Abdel-Hamid, 2013). The authors reported an almost two-time increase in proline concentration in four days after a 6-hour exposure to 40 °C, as compared to the control plants.

Following the 6-hour exposure to 45 °C, in contrast to the SOD activity in the cultivar Surkahn-103, the genotype Bukhara-102 showed a decline, while after eliminating heat stress in 24 hours, the activity significantly ($P \leq 0.05$) rose by 54.1% (Table 1). After the 6-hour exposure to 45 °C, in cotton cultivar Surkhan-103, the activity restoration was 2.7% of the initial level. Presumably, the diverse variations in the enzyme activity of the cotton cultivars suggested specific heat stress adaptation of SOD in various cotton genotypes; an explanation for this could be either an increase in the enzyme degradation or a decline in the synthesis of the enzyme. Furthermore, following the 6-hour exposure to 45 °C, the catalase activity in the cultivar Bukhara-102 decreased and started restoring after the 24-hour exposure to 30 °C, being 111% higher than the same in the cultivar Surkhan-103 (Figure 1). According to Harsh *et al*. (2016), the increase in catalase activity has an association with the thermal stability of the seed leaves of *Vigna aconitifolia*. In their study, 37 genotypes sustained stress, and 28 genotypes demonstrated enhanced CAT activities. The heat stress caused an elevation in CAT activity up to 333.33% in three days after heat stress elimination. Gür *et al*. (2010) also reported similar results in the cotton crop.

Among the large class of antioxidant enzymes, PO, a heme-containing enzyme, catalyzes hydrogen peroxide reduction and participates in various biochemical reactions in plant cells. POs are capable of generating hydrogen peroxide and then hydroxyl radicals via the hydroxyl cycle. POs are also important components in the creation of extension, designed to strengthen cell walls (Passardi *et al*., 2005). The utilization of hydrogen peroxide to non-toxic levels to form water and oxygen is the function of peroxidases by oxidizing an auxiliary substrate such as a phenolic compound. The PO activity appeared to correlate with the plant resistance to stress factors, especially to heat stress. In the promising studies, PO activity increased in both cotton cultivars, with higher activity found in the cultivar Bukhara-102 (Figure 1). However, following the 24-hour exposure to 30 °C, the PO activity declined in Surkhan-103 and Bukhara-102 by 4.4% and 10.8%, respectively. The nonsignificant reduction in the enzyme activity could be due to an increase in the SOD and CAT activity after 24-hour exposure to 30 °C. A significant increase in peroxidase activity was evident in the tolerant genotype of *B. juncea* under high-temperature stress, in contrast to the sensitive

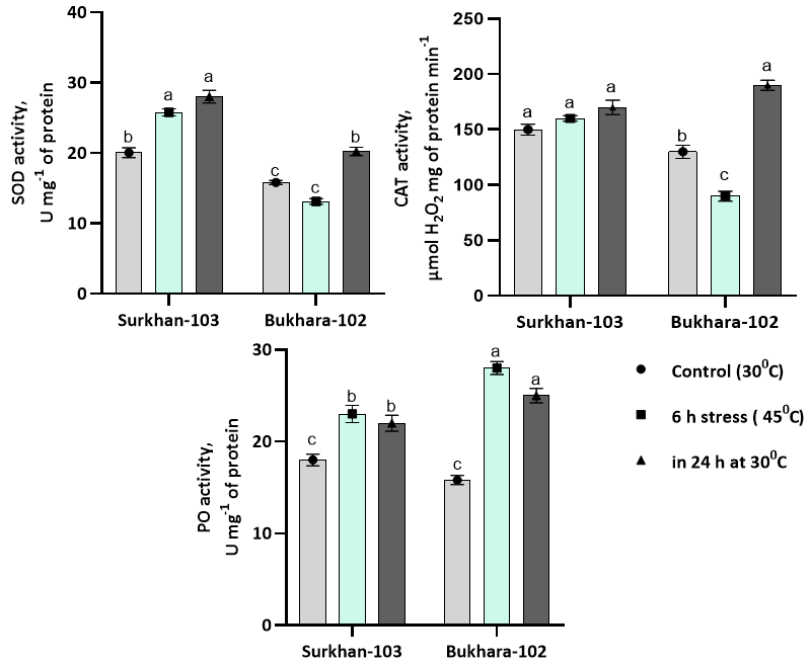

Figure 1. Activities of SOD, CAT, and PO in cotton leaves under 6 hours of exposure to 45 °C and 24 hours of exposure to 30 °C. Data are presented as treatments mean ± SE (n = 3). Means with the same letter(s) on top of the columns do not differ significantly.

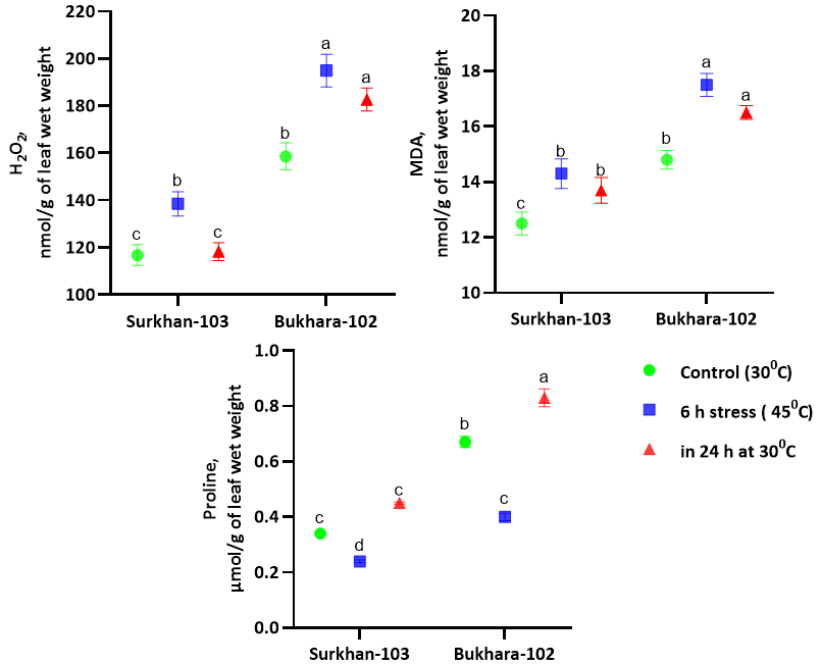

Figure 2. Concentrations of ROS, MDA, and proline in the leaves of cotton seedlings under 6 hours of exposure to high temperature (45 °C) and following the 24-hour exposure to 30 °C. Data are presented as treatments mean ± SE (n = 3). Means with the same letter(s) on top of the columns do not differ significantly.

Table 1. Morphometric indicators of cotton seedlings by exposing to a prolonged heat stress and after restoration.

Cotton cultivars	Underground parts - roots (%)		Above ground part - leaves and stems (%)	
	Inhibition (%) in 6-hour exposure to 45 °C	Restoration (%) in 24-hour exposure to 30 °C	Inhibition (%) in 6-hour exposure to 45 °C	Restoration (%) in 24-hour exposure to 30 °C
Surkhan-103	16.0±0.8[b]	8.1±0.3[e]	10.4±0.5[c]	11.2±0.5[c]
Bukhara-102	19.6±0.95[a]	8.4±0.4[d]	11.4±0.4[d]	7.8± 0.4[d]
$LSD_{0.05}$	1.0	0.6	0.7	0.55

Data are presented as treatments mean ± SE (n = 3). Data followed by same letter are not significantly different by LSD test at $P < 0.05$.

ones (Rani *et al*., 2016). Almeselmani *et al*. (2006) also stated an increase in PO activity under high-temperature stress in wheat genotypes. After the cessation of stress, PO activity decreased considerably but remained above the control level in all genotypes.

In addition to some positive functions of the ROS in plants, the hydrogen peroxide, in particular, can damage the cell biological structures and physiological-biochemical processes in cotton cultivars. The cultivars Surkhan-103 and Bukhara-102 with a 6-hour exposure to 45 °C resulted in an increase in H_2O_2 concentration by 18.5% and 22.0%, respectively (Figure 2). It is a fact that changes in H_2O_2 levels in plant cells experiencing stress conditions serve as an indicator of oxidative stress. The generation of ROS is a fundamental process in higher plants, being utilized for signal transduction in response to environmental changes (Ozden *et al*., 2009). Additionally, Sajid *et al*.'s (2019) findings showed the heat treatment increased hydrogen peroxide levels in various cotton genotypes under field conditions when exposed to 48 °C. Furthermore, research findings demonstrated that H_2O_2 levels rose by 18% at 38 °C and 45 °C in response to heat stress in *G. hirsutum* L. cultivars (Gür *et al*., 2010). Evidently, oxidative reactions trigger plant defense mechanisms by activating high-molecular-weight antioxidants, among which SOD, CAT, and PO play a crucial role (Gür *et al*., 2010).

Similar findings are evident for the MDA concentrations, increasing by 14.4% and 18.2% in the cultivars Surkhan-103 and Bukhara-102, respectively, upon a 6-hour exposure to 45 °C. Meanwhile, upon 24-hour exposure to 30 °C, the MDA concentration emerged to decrease in both genotypes. This could refer to the ROS capacity to initiate lipid peroxidation, resulting in damage to these structures associated with the damage of membrane proteins. The MDA content in seedlings of heat-tolerant lettuce varieties was lower than that of heat-sensitive varieties. In all varieties, MDA levels surged significantly with rising temperatures, with the most pronounced changes observed in the range of 35 °C–40 °C (Han *et al*., 2013).

Proline is a key stress metabolite in plants known for its functions, including the ability to combat stress; however, aspects about the joint action of proline and antioxidant enzymes require further study. Proline concentrations in the cotton cultivars under study were lower than those in the control ones after the 6-hour exposure to 45 °C. Although, following stress elimination after the 24-hour exposure to 30 °C, the proline concentration measured in the fresh leaf weight occurred to significantly ($P \leq 0.05$) restore in the cultivars Surkhan-103 and Bukhara-102 seedlings by 87.5% and 107.5%, respectively (Figure 2). Among the metabolites that accumulate in plants suffering stress conditions, only proline has appeared to exhibit a "quenching" effect on singlet oxygen and hydroxyl radicals formed during the initial hours of stress exposure (Matyusik *et al*., 2002).

Several studies have reported a decrease in the content of MDA, a product of lipid peroxidation, in plant tissues enduring stress conditions due to the influence of proline

(Alia and Mohanty, 2000). The boost in proline seemed beneficial and can induce stress tolerance in cotton (De *et al*., 2000). Higher proline accumulation has been notable in high-yielding cotton varieties, where it actively participates in the detoxification of hydrogen peroxide to maintain optimal physiological conditions under field conditions (Gür *et al*., 2010).

Among the growth stages of plants, the first to incur effects is germination. Heat stress exerts negative impacts on various crops during seed germination, though the ranges of temperatures vary largely on crop species (Johkan *et al*., 2011; Kumar *et al*., 2011). Reduced germination percentage, plant emergence, abnormal seedlings, poor seedling vigor, and lessened radicle and plumule growth of germinated seedlings are major impacts caused by heat stress documented in various cultivated plant species (Toh *et al*., 2008; Piramila *et al*., 2012). The inhibition of seed germination is also well-documented in high temperature, which often occurs through the induction of ABA (Essemine *et al*., 2010). At extreme temperature (45 °C), the rate of germination of wheat strictly stopped and caused death of cells and embryos, for which the seedling establishment rate also lowered (Cheng *et al*., 2009). Plant height, the number of tillers, and total biomass declined in rice cultivars in response to high temperatures (Mitra *et al*., 2008).

Morphometric indicators in the underground and above-ground parts of cotton seedlings after the 6-hour exposure to 45 °C and in 24 hours of exposure to 30 °C also entailed evaluation (Table 1). In cotton seedlings, the root system, as exposed to the heat stress, demonstrated inhibition of the root wet weight up to 20%. In 24 hours of exposure at 30 °C, the root fresh weight restoration was 49.4% and 56.8% in the cultivars Surkhan-103 and Bukhara-102, respectively (Table 1). Restoration in the wet weight of the above-ground parts of the cultivar Bukhara-102 seedlings was 31.5%. The results revealed a correlation with high activities of SOD and CAT and simultaneous reduction in the concentrations of hydrogen peroxide and MDA. Past studies reported significant reductions of morphological criteria in two cotton genotypes after exposure to 40 °C, while the criteria proved to increase in others. The study attributes the variations in the criteria to the rise in the activities of antioxidant enzymes under heat stress (Mohamed and Abdel-Hamid, 2013).

These results also correlated well with high levels of antioxidant enzymes, such as SOD and CAT, as well as a decrease in ROS and MDA content. Similarly, Ding *et al.* (2016) declared that under heat shock at 40 °C for six hours, the morphometric parameters of the investigated varieties significantly decreased, while in other varieties, an increase occurred. The authors attribute the changes in morphometric traits to an upsurge in peroxidase activity under heat treatment (40 °C), which plays an active role in regulating cell elongation.

The hyperthermia effect on the cultivar features

Two cotton cultivars, Surkhan-103 (*G. barbadense* L.) and Bukhara-102 (*G. hirsutum* L.), underwent planting in the special cubicle, 'Phytotron' greenhouse, kept for cotton breeding at the Cotton Breeding, Seed Production, and Agrotechnology Research Institute, Tashkent, Uzbekistan. Inside the greenhouse, the temperature was 15 °C–20 °C higher than outside the complex (Figure 3). Samples used in this experiment comprised seven fine-fiber cotton cultivars, including Surkhan-103, Surkhan-104, Surkhan-105, Surkhan-106, Surkhan-111, Surkhan-14, and Surkhan-16. Likewise, seven medium-fiber cotton cultivars, such as Bukhara-102, Namangan-77, S-6577, S-6585, C-6524, Sultan, and Jarkurgan, and new advanced lines, comprising L-403, L-604, and L-214, were specimens in this research.

In all the 14 cotton cultivars and three advanced lines, a significant ($P \leq 0.05$) low loss of fruit elements could be visible in the fine-fiber cultivars, such as Surkhan-103 (29.7%) and Surkhan-104 (40.0%), as well as in the medium-fiber cultivars, including

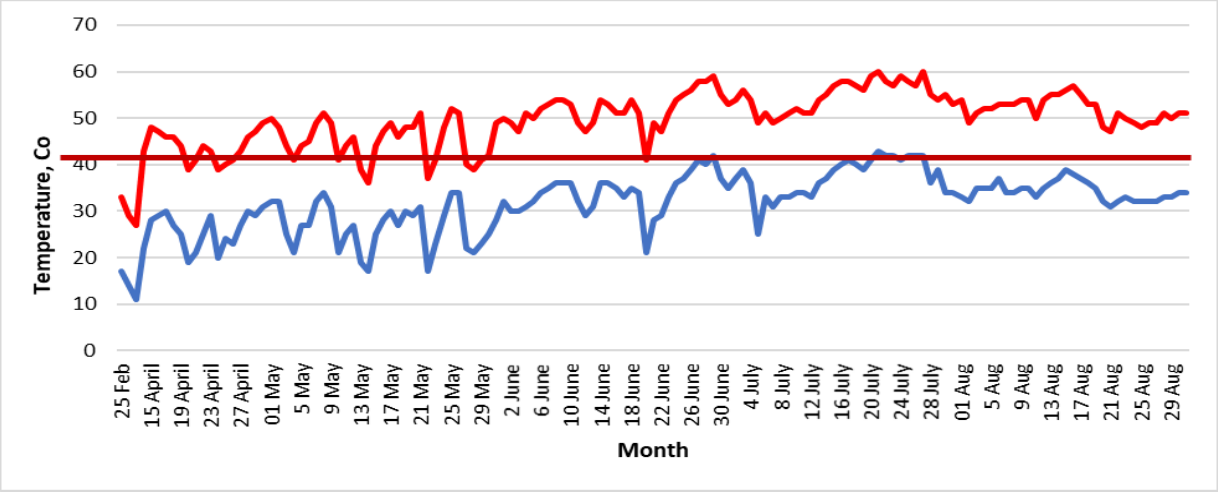

Figure 3. Temperature outside (blue) and inside (red) in the Phytotron greenhouse.

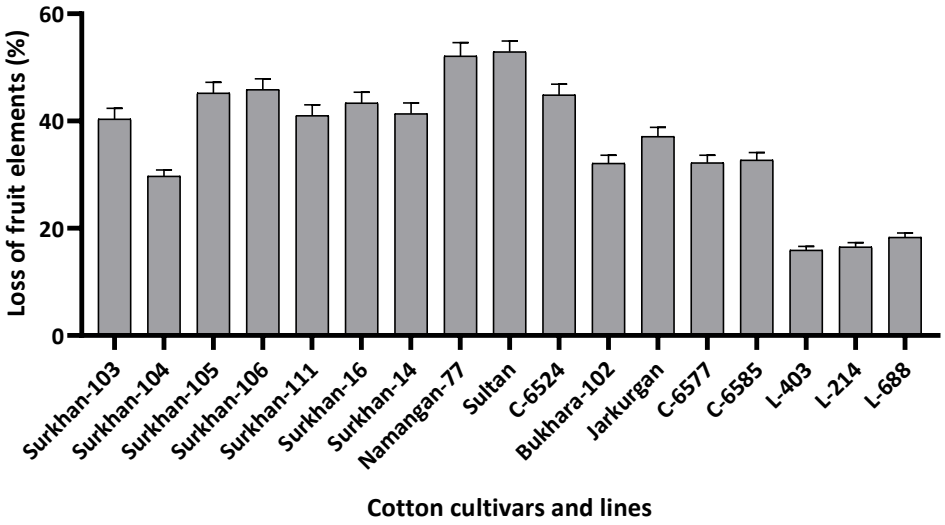

Figure 4. Loss of fruit elements in cotton cultivars and lines (%). Surkhan-103, Surkhan-104, Surkhan-105, Surkhan-106, Surkhan-111, Surkhan-16, Surkhan-14, Namangan-77, Sultan, C-6524, Bukhara-102, Jarkurgan, C-6577, C-6585, L-403, L-214, and L-688.

Bukhara-102 (32.1%), S-6585 (32.7%), and C-6577 (32.2%). One should also note that in new cotton lines L-403, L-604, and L-214, the loss of fruit elements was significantly ($P \leq 0.05$) low, ranging from 15.9% to 18.3%. However, in other cotton cultivars, the fruit elements' loss was remarkably ($P \leq 0.05$) higher, and in cultivars Namangan-77 and Sultan, the losses were 52.1% and 52.9%, respectively (Figure 4).

Thus, based on the biochemical study and morphometric features, cotton cultivars Surkhan-103 and Bukhara-102 achieved classification as thermally stable genotypes. These promising cultivars were noteworthy with the highest activity of SOD, catalase, and PO, which are the inhibitors for ROS and lipid peroxidation. The biochemical findings provided an opportunity to account for an increase in H_2O_2 and MDA concentrations with

the activities of antioxidant enzymes considered as the key marker in assessing the stability of cotton genotypes to the highest temperature stress.

Among cotton cultivars and advanced lines grown under hyperthermia, fine-fiber cultivars Surkhan-104 and Surkhan-103, medium-fiber cultivars Bukhara-102, S-6577, S-6585, and the advanced lines L-688, L-214, and L-403 demonstrated the highest stability. The cultivars with high tolerance to hyperthermia have succeeded in distinction and recommendation to be used in future analytical breeding programs. Furthermore, these findings are valuable for breeders aiming to enhance cotton's thermal tolerance and ensure stable productivity under climate variability.

CONCLUSIONS

Based on biochemical and morphometric findings, the identification of cotton cultivars Surkhan-103 (*G. barbadense* L.) and Bukhara-102 (*G. hirsutum* L.) was successful as heat stress tolerant, with low loss of fruit elements under the highest temperature, as compared to other cultivars and advanced lines. Thus, hyperthermia promoted an increase in H_2O_2 concentration, which correlated with the MDA concentration; it suggested that increases in H_2O_2 and MDA concentrations are the main markers in determining cotton genotypes' stability to high temperature with antioxidant enzymes' activity.

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