Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions

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Coincidence of high temperature with terminal reproductive pheno-stages of cotton is chief constraint to achieve yield potential. This high temperature interfere plant defensive system, physiological process, water relations and lint yield production. In this study, we modulated the detrimental outcomes of heat stress on cotton through the foliar spray of nutrients. Cotton crop was exposed to sub-optimal and supra-optimal thermal regimes for a period of one week at squaring, flowering and boll formation stages under glass house and field conditions. Foliar spray of potassium (K-1.5%), zinc (Zn-0.2%) and boron (B-0.1%) were applied at three reproductive stages one day prior to expose high temperature regimes. High temperature increased lipid membrane damage through increased malondialdehyde (MDA) contents in cotton leaves. High temperature stress also reduced leaf chlorophyll contents, net photosynthetic rate, stomatal conductance, water potential, averaged boll weight (g) and seed cotton yield per plant. Various nutrients variably influenced growth and physiology of heat-stressed cotton plants. Zinc outclassed all other nutrients in increasing leaf SOD, CAT, POX, AsA, TPC activity, chlorophyll contents, net photosynthetic rate, stomatal conductance, water potential, boll weight and seed cotton yield per plant. For example, zinc improved seed cotton yield under supra-optimal thermal regime by 17% and under sub-optimal thermal regime by 12% of glasshouse study while 19% under high temperature sowing dates of field study than the water treated plants under the same temperatures. Conclusively, increasing intensities of temperature adversely affected the recorded responses of cotton and exogenous application of Zn efficaciously alleviated heat induced perturbations. Moreover, exogenous nutrients mediated upregulations in physiochemical attributes induced heat tolerance at morphological level.

Temperature is prophesied to rise by 5.8 °C till 2100 and 2.6 °C up to 2050 owing to global warming. Heat waves along with more number of warm days and nights have been increased in most part of the world. Cotton crop being native of semi-arid regions is highly prone to confront with high temperature at the terminal reproductive stages. Coincidence of high temperature with reproductive stages of cotton is a chief hindrance to accomplish yield potential in sub-continent. Since, temperature rises to 47 °C in May–June while accompanying high humidity in July–August develops a death-valley for cotton influencing all reproductive stages of cotton crop. Heat stress mediated impairment in biosynthesis of antioxidants escalates the synthesis of reactive oxygen species.
crop faced optimal temperatures at all reproductive stages during both years of study and thus considered stage of May-2013 sown crop. Heat stress periods were also observed at boll formation stage of April-2012, May-2012 and April-2013 sown crops experienced heat stress period, while sub optimal temperature prevailed at same stage of May-2013 sown crop. Heat stress periods were also observed at boll formation stage of April-2012 sown crop faced sub optimal conditions. June planted crop faced optimal temperatures at all reproductive stages during both years of study and thus considered optimal or control sowing date.

Maximum temperature ranges of treatment period - one week. At squaring stage, temperature of the May sown crop was raised to supra optimal condition during both years of study. Flowering stage of April-2012, May-2012 and April-2013 sown crops experienced heat stress period, while sub optimal temperature prevailed at same stage of May-2013 sown crop. Heat stress periods were also observed at boll formation stage of April-2012 and April-2013 sown crops while boll formation stage of April-2012 sown crop faced sub optimal conditions. June planted crop faced optimal temperatures at all reproductive stages during both years of study and thus considered optimal or control sowing date.

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**Table 1.** Variable temperatures (thermal regimes) during one week of study under field conditions at three reproductive stages of cotton. Optimal = 34–38 °C, sub optimal = 39–41 °C and supra-optimal up to 46 °C Maximum temperature ranges of treatment period - one week. At squaring stage, temperature of the May sown crop was raised to supra optimal condition during both years of study. Flowering stage of April-2012, May-2012 and April-2013 sown crops experienced heat stress period, while sub optimal temperature prevailed at same stage of May-2013 sown crop. Heat stress periods were also observed at boll formation stage of April-2012 and April-2013 sown crops while boll formation stage of April-2012 sown crop faced sub optimal conditions. June planted crop faced optimal temperatures at all reproductive stages during both years of study and thus considered optimal or control sowing date.

| Growth phases | 2012          | 2013          |
|---------------|--------------|--------------|
|               | April | May | June | April | May | June |
| Squaring (initiation) | 35.0–38.0 °C | 38.5–45.0 °C | 34.0–39.0 °C | 33.0–39.0 °C | 39.0–44 °C | 34.0–37.9 °C |
| Flowering (initiation) | 38.0–44.0 °C | 38.0–45.0 °C | 38.0–45.0 °C | 38.0–45.7 °C | 39.0–41.3 °C | 31.8–37.0 °C |
| Boll formation | 38.0–41.0 °C | 42.5–46.0 °C | 33.0–39.0 °C | 33.9–44.9 °C | 32.0–39.2 °C | 35.5–37.2 °C |

(ROS) and thus induces oxidative stress. Consequently, a cascade of reactions consequence into plethora of ROS that aggravate lipid peroxidation, excessive synthesis of malondialdehyde contents (MDA) and ultimately disrupts the water relations resulting into reduction of growth. High temperature stress mediates incongruities in stomatal movements disrupts the water relations resulting into reduction of growth. Perturbations in biochemical attributes ultimately affect morphological attributes. The optimum temperature for development of boll ranges 25.5 °C–29.5 °C. While, boll weight is adversely affected as temperature rises above 25.5 °C–29.5 °C. Similarly, each 1 °C rise of temperature above day maximum temperature decreases seed cotton yield by 110 kg ha⁻¹. Abiotic stresses along with oxidative stress and nutrient deficiency are the major causes of yield reduction throughout world. Exogenous application of nutrients might prove a potent tool to alleviate deleterious impacts of heat. Moreover, heat triggered decrease in uptake of nutrients from soil under heat stress further enhance the importance of exogenous supply of nutrients. Moreover, foliar applied nutrients produce higher yield and better-quality produce on alkaline calcareous soils. Potassium and zinc are immobile within calcareous soils while B availability is also an issue in these soils. Contrarily, potassium, zinc and boron are required in high quantity during the stress conditions. Potassium, zinc and boron modulates biochemical changes through antioxidant enzymes whereas, the exogenous application of potassium, zinc and boron upregulates the biosynthesis of chlorophyll which ultimately delays senescence enhances their quantity; consequently, improves the photosynthetic rate and the photosynthetic enzymes. Likewise, potassium and zinc mediated regulations in water relations confer heat tolerance by sustaining water and osmotic potential of cell under stress conditions. While, boron availability enhances stomatal opening and thus regulate gaseous exchange under stressed environment.

Therefore, considering the crucial role of macro (K) and micro nutrients (Zn and B) in protecting crops from extensive range of abiotic stresses, exogenous application under stress conditions might prove a potent tool to alleviate adverse impact of stress. The present study compares the potential role of foliar spray of macronutrient (potassium) in photosynthesis, regulation of water relations and stomatal conductance and micronutrients (zinc and boron) in reproduction and antioxidants. These physiochemical regulations might induce tolerance in morphological attributes of cotton crop exposed to different thermal regimes at squaring, flowering and boll formation. Hence, series of glasshouse and field experiments were conducted with objectives to (1) see the effect of different temperature regimes on leaf physiology and lint yield of cotton and (2) reveal the role of macro and micro nutrients (K, Zn and B) for alleviation the impact of high temperature stress.

**Materials and Methods**

**Glasshouse experiment.** The glasshouse experiment was conducted at University of Agriculture Faisalabad (UAF), Pakistan. The experiment was performed during summer 2012. The seed of medium heat tolerant variety (AA-802) was collected from Ali Akbar Enterprises for this study. Soil properties and growth condition were same as have been reported in an earlier study. Four seeds were sown at 2 cm depth had 12 hours pre-soaking in tap water. At four leaf stage of the seedlings, extra plants were thinned left only one plant in each pot. Treatments were comprised of optimal temperature (32/20 ± 2 °C day/night temperature or no stress), sub-optimal temperature (38/24 °C ± 2 °C or medium intensity heat stress) and supra-optimal temperature (45/30 °C ± 2 °C or high intensity heat stress); exogenously applied nutrients viz. water spray (control), foliar spray of K @ 1.5%, foliar spray of Zn @ 0.2% and foliar spray of B @ 0.1%. One day before shifting the pots to medium and high temperature chambers, the plants were sprayed with either of water (control), potassium (1.5%), zinc (0.2%) and boron (0.1%). Foliar concentrations of these nutrients were optimized in the preliminary glasshouse and field experiments (data...
Effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX U mg⁻¹ protein), ascorbic acid (AsA mg⁻¹ FW), total phenolic contents (TPC mg g⁻¹ FW) and malondialdehyde contents (MDA nmol g⁻¹ FW), (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions.

**Table 2.** Effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX U mg⁻¹ protein), ascorbic acid (AsA mg⁻¹ FW), total phenolic contents (TPC mg g⁻¹ FW) and malondialdehyde contents (MDA nmol g⁻¹ FW), (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions. Values are the means of three replications ± SE and variants possessing the same letters are not statistically significant at P < 0.05. Main factors and interaction are significant at P < 0.01. Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.
not shown). All the plants were grown at 32/20 °C up to 30 DAS (before initiation of squaring). After that, pots were divided into 3 sets, each set was consisted of 20 pots which were transferred to growth chambers maintained for different temperature. First set of 20 pots was exposed to heat stress at squaring, 2nd set at flowering and 3rd set at boll formation. Heat stress was imposed for a period of one week at squaring, flowering and boll formation and data recorded were averaged across the three reproductive stages (squaring, flowering and boll formation stages). Samples from the youngest fully expanded leaves were collected immediately after removing the pots from stress, stored in liquid nitrogen and processed to record various attributes. The experiment was conducted using completely randomized design with split arrangement and replicated four times. Varying temperature regimes were imposed in main pots and nutrients were foliar applied in split pots.

**Field experiment.** The field experiments were conducted at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan during 2012 to 2013. The meteorological data were collected by the Meteorological Observatory of the Department of Agronomy, UAE. Treatments were comprised of sowing dates in main plots viz. early April (medium temperature at squaring, flowering and boll formation), early May (high temperature at squaring, flowering and boll formation) and mid-June (optimum temperature at squaring, flowering and boll formation). While, split plot treatments were consisted of foliar sprays of K, Zn and B viz. water spray, foliar spray of K @ 1.5%, foliar spray of Zn @ 0.2% and foliar spray of B @ 0.1%. Different sowing times were

\[
\text{Thermal regimes: Control (water spray) K (1.5%) Zn (0.2%) B (0.1%)}
\]

\[
\begin{align*}
0 & \quad 0.1 & \quad 0.2 & \quad 0.3 & \quad 0.4 & \quad 0.5 & \quad 0.6 & \quad 0.7 & \quad 0.8 & \quad 0.9 & \quad 1 \\
32/20 \pm 2°C & 45/30 \pm 2°C & 38/24 \pm 2°C
\end{align*}
\]

Figure 2. Effect of different thermal regimes and nutrients’ spray on chlorophyll contents (a + b) (mg g\(^{-1}\)FW), net photosynthetic rate-Pn (µmol m\(^{-2}\) sec\(^{-1}\)), stomatal conductance (Gs m mol m\(^{-2}\) s\(^{-1}\)), leaf water potential (−MPa) and leaf osmotic potential (−MPa) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions.
**Table 3.** Effect of different thermal regimes and nutrients’ spray on chlorophyll contents (a + b) (mg g \(^{-1}\)FW), net photosynthetic rate-Pn (\(\mu\)mol m\(^{-2}\) sec\(^{-1}\)), stomatal conductance (Gs m mol m\(^{-2}\) sec\(^{-1}\)), leaf water potential (\(-\)MPa) and leaf osmotic potential (\(-\)MPa) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions. Values are the means of three replications \(n = 4\) ± SE and variants possessing the same letters are not statistically significant at \(P < 0.05\). Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.

| Thermal regimes | Nutrients | Chlorophyll a | Chlorophyll b | Pn       | Gs       | Water Potential | Osmotic potential |
|-----------------|-----------|---------------|---------------|----------|-----------|-----------------|------------------|
| 32/20°C         | Control   | 1.34 b ± 0.070| 0.46 a ± 0.27 | 26.69 ± 0.63| 0.79 a ± 0.042| 0.46 a ± 0.041| 0.68 a ± 0.034  |
|                 | Potassium | 1.51 a ± 0.073| 0.48 a ± 0.30 | 26.38 ± 0.67| 0.79 a ± 0.040| 0.46 a ± 0.040| 0.68 a ± 0.032  |
|                 | Zinc      | 1.50 a ± 0.076| 0.49 a ± 0.32 | 26.20 ± 0.57| 0.78 a ± 0.035| 0.44 a ± 0.039| 0.67 a ± 0.035  |
|                 | Boron     | 1.37 b ± 0.068| 0.48 a ± 0.26 | 26.04 ± 0.12| 0.77 ± 0.036  | 0.44 a ± 0.038 | 0.66 ± 0.031    |
|                 |           | 0.80 c ± 0.051| 0.28 ± 0.20  | 16.74 ± 0.37| 0.50 c ± 0.024| 0.76 a ± 0.070 | 1.10 ± 0.052    |
|                 | Potassium | 1.18 a ± 0.062| 0.41 ± 0.23  | 22.66 ± 0.40| 0.71 ± 0.037  | 0.56 c ± 0.052 | 0.81 ± 0.040    |
|                 | Zinc      | 1.25 a ± 0.067| 0.39 ± 0.21  | 22.11 ± 0.37| 0.70 a ± 0.033| 0.57 c ± 0.055 | 0.80 ± 0.038    |
|                 | Boron     | 1.07 b ± 0.055| 0.33 ± 0.19  | 19.79 ± 0.29| 0.60 b ± 0.031| 0.68 b ± 0.064 | 0.91 b ± 0.045  |
|                 | LSD       | 0.080         | 0.023        | 1.38      | 0.032      | 0.021           | 0.031           |

**Table 4.** Effect of different thermal regimes and nutrients’ spray on averaged boll weight (g) and seed cotton yield per plant (g) of cotton crop under glass house conditions. Values are the means of three replications \(n = 4\) ± SE and variants possessing the same letters are not statistically significant at \(P < 0.05\). Main factors and interaction are significant at \(P < 0.01\). Lettering is done separately for each thermal regime using the LSD of the interaction between thermal

| Thermal regimes | Nutrients | Boll Weight (g) | SCY       |
|-----------------|-----------|----------------|-----------|
| 32/20°C         | Control   | 3.70 ± 0.15    | 83.22 ± 3.1|
|                 | Potassium | 4.36 ± 0.22    | 84.99 ± 2.9|
|                 | Zinc      | 4.25 ± 0.20    | 85.05 ± 4.1|
|                 | Boron     | 4.13 ± 0.18    | 85.47 ± 4.2|
|                 | LSD       | 0.20           | 2.99       |

referred as thermal regimes. The varying temperature was recorded at squaring, flowering and boll formation stages. Three sowing times (April 2, May 3 and June 17 during 2012 and April 4, May 2 and June 19 during 2013) were selected based on previous five years’ climate data. Sowing of both experiments was done on sandy clay loam soil at times as per treatments during 2012 and 2013. Seed of cotton variety (AA- 802) was collected from Ali Akbar Enterprises during both years of study. Crop was planted with manual dibbling having 75 cm apart ridges and plant to plant distance was 30 cm. Weeds were controlled by two hoeing i.e. at squaring (35 days after planting) and at flowering (60 days after planting) while the sucking insects and boll worms were controlled with insecticides. Nine irrigations were applied as per crop requirement keeping in view the reproductive stages having heat stress at different sowing dates to avoid the drought stress during heat stress periods. The data of physiological parameters were recorded across the three reproductive stages of cotton i.e. squaring, flowering and boll formation. June thermal regime (late sown crop) was considered control, as it provided optimal temperature at all reproductive stages, while April (early sowing) and May sown crops were experienced sub and supra-optimal temperatures at three reproductive stages (Table 1). The experiment was laid out in randomized complete design with split treatment structure having three replications. Sowing dates were randomized in main and exogenous nutrients in split plots.
Figure 3. Effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) U mg⁻¹ protein and ascorbic acid (AsA mg g⁻¹ FW) contents (averaged across squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013. Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at P < 0.05. Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.

| Thermal regimes          | Nutrients   | SOD 2012 | SOD 2013 | CAT 2012 | CAT 2013 | POX 2012 | POX 2013 | AsA 2012 | AsA 2013 |
|--------------------------|-------------|----------|----------|----------|----------|----------|----------|----------|----------|
| **Optimal regimes of sowing dates** |             |          |          |          |          |          |          |          |          |
| Control                  |             | 41.33 cd ± 3.8 | 39.42 bc ± 3.5 | 58.11 cd ± 5.60 | 59.49 cd ± 5.60 | 37.35 ab ± 3.6 | 35.30 b ± 3.0 | 147.94 b ± 13.40 | 145.45 b ± 14.0 |
| Potassium (1.5%)         |             | 47.55 b ± 4.4 | 47.69 a ± 4.3 | 88.01 b ± 7.08 | 93.48 b ± 7.08 | 45.04 a ± 3.9 | 49.49 a ± 4.6 | 204.21 a ± 18.70 | 196.40 a ± 17.0 |
| Zinc (0.2%)              |             | 58.73 a ± 5.3 | 57.35 a ± 5.1 | 140.86 a ± 10.70 | 144.94 a ± 10.70 | 47.62 a ± 4.1 | 52.47 a ± 4.5 | 215.12 a ± 19.40 | 206.47 a ± 19.6 |
| Boron (0.1%)             |             | 44.10 bc ± 4.1 | 43.12 ab ± 3.7 | 73.11 bc ± 5.60 | 76.42 bc ± 5.60 | 39.67 a ± 3.7 | 38.07 b ± 3.6 | 192.34 a ± 17.40 | 187.12 a ± 19.20 |
| **Supra-optimal of sowing dates** |             |          |          |          |          |          |          |          |          |
| Control                  |             | 56.04 d ± 4.9 | 54.04 d ± 4.9 | 81.81 d ± 5.90 | 78.22 d ± 5.90 | 46.86 c ± 4.0 | 41.85 c ± 4.0 | 164.37 c ± 15.28 | 158.87 c ± 14.50 |
| Potassium (1.5%)         |             | 86.66 b ± 7.8 | 84.34 b ± 8.1 | 230.87 b ± 10.30 | 221.95 b ± 10.30 | 93.14 a ± 8.3 | 86.44 a ± 7.6 | 312.83 a ± 30.20 | 298.17 a ± 27.60 |
| Zinc (0.2%)              |             | 110.28 a ± 10.1 | 105.12 a ± 9.9 | 280.19 a ± 10.66 | 264.68 a ± 10.66 | 101.56 a ± 9.7 | 91.34 a ± 8.2 | 325.01 a ± 31.50 | 310.61 a ± 30.14 |
| Boron (0.1%)             |             | 69.17 c ± 6.3 | 66.75 c ± 6.1 | 161.45 c ± 4.07 | 153.64 c ± 4.07 | 74.61 a ± 6.6 | 71.32 b ± 6.5 | 239.03 a ± 22.60 | 221.04 b ± 20.90 |
| **Sub-optimal of sowing dates** |             |          |          |          |          |          |          |          |          |
| Control                  |             | 46.21 cd ± 4.3 | 43.79 cd ± 3.6 | 73.17 d ± 5.47 | 70.24 cd ± 5.47 | 41.89 bc ± 4.0 | 38.08 c ± 3.2 | 154.05 b ± 14.80 | 155.11 b ± 13.50 |
| Potassium (1.5%)         |             | 56.54 b ± 5.1 | 52.63 b ± 4.9 | 148.08 b ± 6.53 | 122.20 b ± 6.53 | 62.00 a ± 5.2 | 67.92 a ± 5.4 | 221.52 a ± 21.40 | 211.13 a ± 21.30 |
| Zinc (0.2%)              |             | 67.04 a ± 6.5 | 64.42 a ± 6.2 | 192.29 a ± 11.47 | 168.10 a ± 11.47 | 65.23 a ± 5.8 | 72.38 a ± 6.5 | 225.64 a ± 21.0 | 223.88 a ± 20.80 |
| Boron (0.1%)             |             | 51.56 bc ± 4.5 | 49.21 bc ± 4.8 | 112.44 c ± 7.17 | 95.41 c ± 7.17 | 50.43 b ± 4.6 | 51.00 b ± 4.2 | 201.08 a ± 18.40 | 200.43 a ± 17.80 |
| LSD                      |             | 10.46      | 11.04     | 27.83     | 25.86     | 11.00     | 7.80      | 37.93     | 33.19     |

Table 5. Effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX U mg⁻¹ protein) and ascorbic acid (AsA mg g⁻¹ FW) contents (averaged across squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013. Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at P < 0.05. Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.
Figure 4. Effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX U mg⁻¹ protein), ascorbic acid (AsA mg g⁻¹ FW), total phenolic contents (TPC mg g⁻¹ FW) and malondialdehyde contents (MDA nmol g⁻¹ FW), (averaged across of squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013.

Table 6. Effect of different thermal regimes and nutrients’ spray on total phenolic contents (TPC mg g⁻¹ FW), malondialdehyde contents (MDA nmol g⁻¹ FW) and chlorophyll contents (a + b) (mg g⁻¹FW) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013. Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at P<0.05. Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.
**Biochemical assays.** Leaves samples weighing 0.5 g were extracted in with 10 ml of phosphate buffer (pH 7.8) for the extraction of enzymes. The supernatant was used for enzyme determination after centrifuge and the residues were discarded. The extracted material was stored at 4 °C.  

Superoxide dismutase contents were determined by method. Superoxide dismutase was quantified as enzymes units that inhibited photo reduction of nitrobluetetrazolium (NBT) and recorded the absorbance at 470 nm. While, CAT was measured as enzymes units that converted H2O2 to H2O and O2 using the protocol as described by Liu. The reaction mixture [50 mM phosphate buffer (pH 7) + 5.9 mM H2O2] was mixed with 0.1 mL enzyme extract and read the absorbance at 240 nm. Peroxidase contents were determined using method as given by. Peroxidase was quantified as units of enzymes that oxidized guaiacol. The reaction mixture was comprised of 50 mM phosphate buffer (pH 5) + 40 mM H2O2 + 20 mM guaiacol and 0.1 mL of enzyme extract per each sample. The absorbance was recorded at wavelength of 470 nm.

For the estimation of ascorbic acid, 900 µL distilled H2O + 100 µL sample extract + 1 mL dichlorophenol-indophenol + 100 µL 0.1% Meta H2PO4 were mixed in a test tube and absorbance was recorded at 520 nm. Folin-Ciochette (FC) reagent method was used for the determination of TPC. Leaves samples of 0.5 g weight were extracts with 80% acetone (10 mL) and centrifuged. Enzyme extract (20 µL) + FC-reagent (100 µL) + 1.5 mL water were mixed in a cuvette and placed for 30 minutes. Then, added 700 mM Na2CO3 and incubated at room temperature for period of 2 hours. The absorbance was taken at 765 nm having 200 µL sample in each well. MDA contents in cotton leaves were determined according following the procedure as adapted by. Leaf sample (0.5 g) was homogenized in 10 ml of 0.1% trichloroacetic acid (TCA) solution and centrifuged at 12000 × g for 15 minutes. For each mL of extract 4.5 mL of thiobarbituric acid (0.5%) was used with the reaction mixture and heated at 95°C for 30 min and cooled. The absorbance was taken at 532 and 600 nm and MDA concentration was determine using formula:

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\text{MDA level (nmol)} = \frac{\Delta (A532 \text{ nm} - A600 \text{ nm})}{156 \times 10^5} A
\]

where A is Absorption coefficient with the value of 156 mm−1 cm−1.

**Observations.**
yield per plant (g) of cotton crop under field conditions during 2012 and 2013. Values are the means of three

Table 7. Effect of different thermal regimes and nutrients’ spray on net photosynthetic rate-Pn (μmol m\(^{-2}\) sec\(^{-1}\)), FW, stomatal conductance (Gs m mol m\(^{-2}\) s\(^{-1}\)), leaf water potential (−MPa) and leaf osmotic potential (−MPa) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013. Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at P < 0.05. Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.

| Nutrients | Control | Potassium (1.5%) | Zinc (0.2%) | Boron (0.1%) |
|-----------|---------|------------------|-------------|-------------|
| Pn 2012   | 27.43 ± 2.4 | 26.83 ± 2.1 | 26.05 ± 2.3 | 26.01 ± 2.0 |
| Pn 2013   | 27.27 ± 2.5 | 26.18 ± 2.2 | 26.07 ± 2.1 | 26.20 ± 2.4 |
| Gs 2012   | 0.81 ± 0.076 | 0.79 ± 0.073 | 0.80 ± 0.071 | 0.80 ± 0.077 |
| Gs 2013   | 0.81 ± 0.075 | 0.81 ± 0.077 | 0.80 ± 0.075 | 0.80 ± 0.074 |
| Water Potential 2012 | 0.45 ± 0.039 | 0.45 ± 0.035 | 0.42 ± 0.040 | 0.43 ± 0.038 |
| Water Potential 2013 | 0.46 ± 0.041 | 0.44 ± 0.038 | 0.42 ± 0.036 | 0.47 ± 0.043 |
| Osmotic Potential 2012 | 0.70 ± 0.065 | 0.70 ± 0.062 | 0.70 ± 0.069 | 0.69 ± 0.060 |
| Osmotic Potential 2013 | 0.69 ± 0.062 | 0.69 ± 0.065 | 0.68 ± 0.059 | 0.68 ± 0.061 |

Table 8. Effect of different thermal regimes and nutrients’ spray on averaged boll weight (g) and seed cotton yield per plant (g) of cotton crop under field conditions during 2012 and 2013. Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at P < 0.05. Lettering is done separately for each thermal regime using the LSD of the interaction between thermal regimes and nutrients’ spray.

| Nutrients | Control | Potassium (1.5%) | Zinc (0.2%) | Boron (0.1%) |
|-----------|---------|------------------|-------------|-------------|
| Boll weight (g) 2012 | 3.53 a ± 0.31 | 4.13 a ± 0.38 | 4.05 a ± 0.40 | 26.92 a ± 2.2 |
| Boll weight (g) 2013 | 3.64 b ± 0.32 | 4.24 a ± 0.39 | 4.16 a ± 0.37 | 22.10 ± 1.9 |
| SCY 2012 | 87.60 b ± 8.4 | 101.34 a ± 9.9 | 98.44 a ± 9.2 | 6.63 ± 0.057 |
| SCY 2013 | 85.60 b ± 8.1 | 99.34 a ± 9.4 | 97.62 a ± 9.2 | 0.53 ± 0.049 |

Chlorophyll contents. Cotton leaves (0.5 g) were ground in 10 ml of 80% cold acetone and the tubes were stored in dark at 20°C overnight, indicating minor modifications of previously described method13. The mixture was filtered through a Whatman No 1. A blank with 80% acetone was run; the measurements were taken at 645 and 663 nm through a spectrophotometer. The chlorophyll contents were calculated from the formula:
Chla (mg/g) = \[\frac{12.7 (OD 663) - 2.69 (OD 645)}{1000} \times W\]

Chlb (mg/g) = \[\frac{22.9 (OD 645) - 4.68 (OD 663)}{1000} \times W\]

where W is the weight of leaf sample while V is the volume of sample used in spectrophotometer (U-2001, Hitachi, Japan).

Net photosynthetic rate and stomatal conductance. Net photosynthetic rate and stomatal conductance was determined at three reproductive stages of cotton crop through a portable infrared gas analyzer (LClAnalyser having Broad Head, Part Number LCI-002/B with Serial Number 32455). The Pn was measured at each reproductive stage after 3 days of spray between 10:00 a.m. to 12:00 p.m. on fully expanded young leaves.

Water relations. Leaf samples (Leaf water and osmotic potential) were collected at pre-dawn (6:00 h) as previously described. Leaf water potential was determined through Scholander type pressure chamber (ARIMAD 2, Korea) following methodology as described instantly after sampling. While, leaves were stored at −20 °C for a period of one week, then thawed, extracted sap and determined the osmotic potential with the help of osmometer (Osmomat 030).

Agronomic attributes. Ten plants were randomly selected in each experimental unit of filed study while five plants were selected from five random pots of optimal, sub and supra-optimal thermal regimes of glass house study. Averaged boll weight was noted by dividing total seed cotton yield per plant with total number of bolls. While, seed cotton yield was weighed separately for each plot/pot and converted to per hectare yield from each plot.

Statistical analysis. Analysis of variance was employed to determine significance (F-test) of heat and foliar nutrients. While, means of treatments were compared using least significant difference test (p ≤ 0.05). Correlation among the varying response variables was computed using means of treatments calculated across the three blocks. Strength, type and significance of correlation was determined using STATISTIX 8.1 software (Analytical Software, Tallahassee, Florida, USA). Number of pairs of observations (n) to determine correlation were 36 (replications × main plots × sub plots). Figures were developed using MS excel-2016.

Results

Green house experiment. Significant interaction of heat and foliar nutrients was recorded for all the studied attributes. Supra optimal regime followed by sub optimal regime triggered increase in antioxidants, MDA and decrease in chlorophyll contents, photosynthetic rate, gaseous exchange components, water relations, boll weight and seed cotton yield over the optimal temperature regime. Foliar applied ‘0.2% Zn’ depicted outstanding results
regarding the alleviation of adverse impacts of heat, followed by ‘1.5% K’ and ‘0.1% B’ for all the studied attributes (Tables 2–4, Figs 1–3).

Superoxide dismutase contents were improved by 46% and 25% while catalase contents were increased by 61% and 29% when compared the controls of supra and sub-optimal thermal regimes with the control of optimal thermal regime averaged across of three reproductive stages. Similarly, POX, AsA and TPC contents were increased under sub and supra-optimal thermal regimes. Biosynthesis of SOD was enhanced by 32% and 56% with ‘0.2% Zn’ compared to water spray under sub and supra-optimal thermal regimes. Similarly, ‘0.2% Zn’ instigated improvements in biosynthesis of CAT by 60% and 73% under sub and supra-optimal temperature regime than their respective water treated plants. The SOD and CAT contents were also increased under optimal thermal regime but the effect was more pronounced under sub and supra-optimal thermal regimes. Whereas, POX was up-regulated by 31, 32 and 52% under optimal, sub optimal and supra optimal temperature regimes, respectively (Table 2, Fig. 1).

The Chlorophyll a and b contents were reduced by 15% and 66% under the controls (water treated plants) of sub and supra-optimal thermal regimes when compared with water treated plants of optimal thermal regime. Net photosynthetic rate was reduced by 20% and 60% when compared the water treated plants of sub and supra-optimal thermal regimes with water treated plants of optimal thermal regime (averaged across of three reproductive stages). Similarly, stomatal conductance and water potential were reduced while osmotic potential was increased under sub and supra optimal thermal regimes.

The comparative improvements in chlorophyll a, b contents, Pn and Gs owing to ‘0.2% Zn’ with respect to water spray were statistically higher under sub and supra optimal thermal regimes. For example, Zn improved Chlorophyll a content by 23% and 46% under sub and supra optimal thermal regimes than water treated plants.
Similarly, zinc also improved chlorophyll $b$ contents, $Pn$, $Gs$ and water potential under sub and supra optimal thermal regimes. (Table 3, Fig. 2).

Although, the seed cotton yield (SCY) was reduced by 66% and 23% in the controls of supra and sub-optimal thermal regimes than the control of optimal thermal regime. The similar reduction was found for averaged boll weight. The foliar spray of three nutrients (K, Zn and B) improved SCY by 21%, 16% and 7% in the high temperature regime than water treated plants. Likewise, the nutrients improved the averaged boll weight under high temperature regime (Table 4, Fig. 3).

Field experiment. Supra optimal temperature regimes were relatively more detrimental, and it was followed by sub optimal temperature regimes (Table 1). While, exogenously applied nutrients depicted significant improvements compared to water spray (control). However, relatively more promising results were obtained with '0.2% Zn', followed by '1.5% K', '0.1% B' and water spray. The recorded improvements by the application of exogenous nutrients differ significantly under varying temperature regimes. (Tables 5–8, Figs 4–6).

In the controls of supra and sub optimal thermal regimes of April and May sown crops, the SOD and CAT contents were increased by 37%, 36% and 22%, 11% (averaged across both years of study and of three developmental stages) than the water treated plants of optimal thermal regime. Significantly higher activities of SOD, CAT, POX, AsA and TPC with '0.2% Zn' compared to foliar spray of other nutrients were quantified under all temperature regimes for most of cases over the years. However, '0.2% Zn' mediated improvements in biosynthesis of SOD compared to water spray were 32% under sub optimal and 49% under supra optimal temperature regimes averaged across both years of study. Likewise, CAT, POX, AsA and TPC contents were improved by foliar spray of zinc under sub and supra optimal thermal regimes over water treated plant (Tables 5 and 6, Fig. 4).

Statistically significant decrease in MDA contents were observed with '0.2% Zn' and '1.5% K' compared to other exogenous treatments under varying temperature regimes. However, remarkable change in biosynthesis of MDA was recorded with '0.2% Zn' compared to other sprays. Foliar spray of '0.2% Zn' instigated downregulation in
MDA contents compared to water spray were 52% under sub optimal and 59% under supra optimal temperature regimes averaged across both years of study (Table 6, Fig. 4).

Chlorophyll a, b contents were reduced by 66%, 51% and 16%, 13%, respectively in the controls of supra and sub-optimal thermal regimes than the controls of optimal thermal regime averaged across of both years and of three development stages. Statistically alike and significantly more chlorophyll a and b biosynthesis was quantified with ‘0.2% Zn’ and ‘1.5% K’ compared to other foliar sprays under supra optimal temperature regime (Table 6, Fig. 5).

The Pn was reduced by 47% and 13% under the controls of supra and sub-optimal thermal regimes than the control of optimal thermal regime averaged during both years of study and of three reproductive stages. Significantly more net photosynthetic rate, stomatal conductance, water potential and less osmotic potential were...
and a positive relation with CAT, SOD and with chlorophyll a, b contents (Table 10). Similarly, Pn and SCY have significantly positive relationship with CAT, SOD and with chlorophyll a and b contents (Table 10).

Regression and correlation of studied components under glasshouse and field conditions during 2012 and 2013. The three nutrients (K, Zn and B averaged across) increased SCY under April and May thermal regimes by 15% and 17% than water spray averaged across during both years of study (Table 8, Fig. 6).

Table 10. Correlation between Chlor.a/b, Pn, CAT, SOD and SCY under field conditions during 2012 and 2013. Chlor a (Chlorophyll a), Chlor b (Chlorophyll b), CAT (Catalase), SOD (Superoxide dismutase), Pn (Net photosynthetic rate) and SCY (Seed cotton yield). *Correlation is significant at 0.01 levels. **Correlation is significant at 0.05 levels. n (number of pairs of observations) = 36.

| Parameters | Years | CAT   | Chl a | Chl b | Pn   | SCY |
|------------|-------|-------|-------|-------|------|-----|
| Chl a      | 2012  | 0.74**|       |       |      |     |
|            | 2013  | 0.89**|       |       |      |     |
| Chl b      | 2012  | 0.67**| 0.94**|       |      |     |
|            | 2013  | 0.77**| 0.75**|       |      |     |
| Pn         | 2012  | 0.85**| 0.90**| 0.63**|      |     |
|            | 2013  | 0.72**| 0.86**| 0.68**|      |     |
| SCY        | 2012  | 0.60**| 0.70**| 0.66**| 0.80**|     |
|            | 2013  | 0.67**| 0.74**| 0.59**| 0.89**|     |
| SOD        | 2012  | 0.87**| 0.65**| 0.71**| 0.52**| 0.63**|
|            | 2013  | 0.89**| 0.65**| 0.84**| 0.79**| 0.69**|

discussed with foliar ‘0.2% Zn’ and ‘1.5% K’ under supra optimal temperature regime over two years of experimentation (Table 7, Fig. 5).

In glasshouse, Chlorophyll a and b have significant positive relationship with each other (P < 0.05 and P < 0.01) and with CAT, SOD, Pn and SCY (P < 0.05 and P < 0.01). Similarly, Pn and SCY have significantly positive relationship with CAT, SOD and with chlorophyll a and b contents (Table 9). While in filed conditions, Chlorophyll a and b have significant positive relationship with each other (P < 0.05 and P < 0.01) and with CAT, SOD, Pn and SCY (P < 0.05 and P < 0.01). Similarly, Pn and SCY have significantly positive relation with CAT, SOD and with chlorophyll a, b contents (Table 10).

Regression and correlation of studied components under glass house conditions.

Discussion

Medium high to high temperature regimes influenced cotton crop physiology and the yield45. Reactive oxygen species (ROS) affected the membranes of each organelle for example the integrity of chloroplast/photosynthetic machinery36,67. A balance is required for ROS and antioxidants for the normal functions of plant defensive system. The oxidative stress causes the blockage of nutrients channels5,38. The high temperature regimes (45/30 °C) of glasshouse, Chlorophyll a and b have strong negative relationship with POX, AsA, TPC and with stomatal conductance while water relations have strong positive relationship with stomatal conductance (Fig. 7).

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Regression and correlation of studied components under glass house conditions.
In this study, foliar spray of potassium, and zinc upsurgs the boll weight and seed cotton yield even in K and Zn enriched soil\textsuperscript{2,3,4}. This may be the outcome of the higher production of carbohydrates\textsuperscript{5}. Under glass house and field studies, the chlorophyll (a and b) contents, Pn, stomatal conductance and antioxidants showed positive correlation with each other and with SCY while MDA showed strong negative relation with these parameters as reported by\textsuperscript{6}.

**Conclusion**

High temperature stress at three reproductive stages of cotton crop caused yield reduction which due to lower boll weight that is associated to less chlorophyll contents and impaired photosynthesis. Exogenous application of macro and micro nutrients (K-1.5%, Zn-0.2% and B-0.1%) ameliorated the high temperature impact on cotton crop. These nutrients especially K, Zn and followed by B up-regulated the antioxidant enzymes (SOD, POX, CAT, AsA, phenolics and MDA), improved chlorophyll contents, net photosynthetic rate, water relations and seed cotton yield.

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**Author Contributions**

Muhammad Sarwar, Muhammad Farrukh Saleem conducted the experiment and wrote the paper; Muhammad Rizwan, Shafaqat Ali supervised the study; Najeeb Ullah, Muhammad Rizwan Shahid, Saud A. Alamri; organized and analyzed the data Mohammed Nasser Alyemeni, Parvaiz Ahmad, provided the technical support and chemicals required for the experiment.

**Additional Information**

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