

OPEN

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

Muhammad Sarwar¹, Muhammad Farrukh Saleem², Najeeb Ullah³, Shafaqat Ali⁴, Muhammad Rizwan⁴, Muhammad Rizwan Shahid⁵, Mohammed Nasser Alyemeni⁶, Saud A. Alamri⁶ & Parvaiz Ahmad^{6,7}

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 warming¹. Heat waves along with more number of warm days and nights have been increased in most part of the world². Cotton crop being native of semiarid regions is highly prone to confront with high temperature at the terminal reproductive stages³. 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 crop⁴. Heat stress mediated impairment in biosynthesis of antioxidants escalates the synthesis of reactive oxygen species

¹Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. ²Department of Agronomy, University of Agriculture Faisalabad, 38040, Faisalabad, Pakistan. ³Queensland Alliance for Agriculture and Food Innovation | Centre for Plant Science, University of Queensland Wilsonton Heights, Toowoomba, QLD 4350, Australia. ⁴Department of Environmental Sciences and Engineering, Government College University Allama Iqbal Road, 38000, Faisalabad, Pakistan. ⁵Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, 38000, Pakistan. ⁶Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. ⁷Department of Botany, S.P. College, Maulana Azad Road, Srinagar, Jammu and Kashmir, 190001, India. Correspondence and requests for materials should be addressed to S.A. (email: shafaqataligill@yahoo.com) or P.A. (email: parvaizbot@yahoo.com)

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	43.5–45.0 °C	33.0–38.0 °C	37.0–45.7 °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

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 May-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.

(ROS) and thus induces oxidative stress^{4,5}. Consequently, a cascade of reactions consequence into plethora of ROS that aggravate lipid peroxidation, excessive synthesis of malondialdehyde contents (MDA)⁶ and ultimately disruption in stability of membranes. Henceforth, the biosynthesis of chlorophyll contents decreases while degradation aggravates under heat triggered oxidative stress⁷. Whereas, impairment in biosynthesis of chlorophyll ultimately down regulates photosynthesis, translocation of assimilates to reproductive organs and accelerates senescence^{8,9}. Moreover, heat stress also disrupts stomatal movement and ultimately consequence into poor gaseous exchange. Cotton leaves depict optimum stomatal conductance in temperature ranges 28–30.1 °C¹⁰ which starts to decline as temperature rises to 36 °C. Contrarily, as the temperature rises above 40 °C, stomata remain open and photosynthesis is impaired even if soil is well watered. Concurrently, high temperature stress mediated incongruities in stomatal movements disrupts the water relations resulting into reduction of growth^{11,12}. 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 °C¹³. Similarly, each 1 °C rise of temperature above day maximum temperature decreases seed cotton yield by 110 kg ha⁻¹¹⁴. Abiotic stresses along with oxidative stress and nutrient deficiency are the major causes of yield reduction throughout world¹⁵. Exogenous application of nutrients might prove a potent tool to alleviate deleterious impacts of heat¹⁶. 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 soils¹⁷. Potassium and zinc are immobile within calcareous soils while B availability is also an issue in these soils^{18–20}. Contrarily, potassium, zinc and boron are required in high quantity during the stress conditions. Potassium, zinc and boron modulates biochemical changes through antioxidant enzymes^{21–23} whereas, the exogenous application of potassium, zinc and boron up regulates the biosynthesis of chlorophyll which ultimately delays senescence enhances their quantity; consequently, improves the photosynthetic rate and the photosynthetic enzymes²⁴. 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^{25,26}.

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 to (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 study⁴. 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

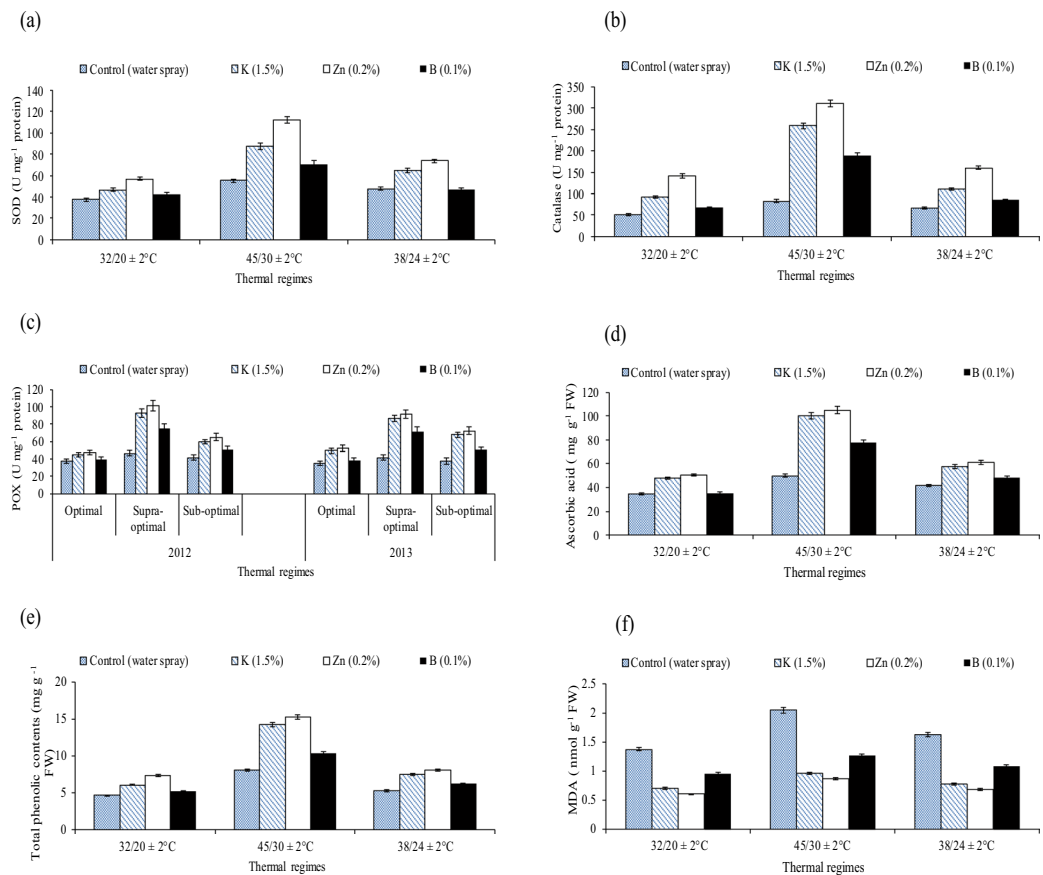


Figure 1. Effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX U mg⁻¹ protein), ascorbic acid (AsA mg g⁻¹ FW), total phenolic contents (TPC mg g⁻¹ FW) and malondialdehyde contents (MDA nmol g⁻¹ FW), (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions.

Thermal regimes	Nutrients	SOD	CAT	POX	ASA	TPC	MDA
32/20 °C	Control	37.71 d ± 2.0	51.33 d ± 3.13	34.89 c ± 0.52	137.92 d ± 8.63	4.63 d ± 0.52	1.37 a ± 0.65
	Potassium (1.5%)	46.31 b ± 2.5	91.85 b ± 5.97	47.96 b ± 0.74	195.58 b ± 10.25	6.06 b ± 0.30	0.70 c ± 0.40
	Zinc (0.2%)	56.71 a ± 3.0	140.39 a ± 8.82	50.96 a ± 0.98	207.39 a ± 12.35	7.27 a ± 0.41	0.60 d ± 0.35
	Boron (0.1%)	42.78 bc ± 2.3	68.57 c ± 4.35	35.23 c ± 0.56	177.94 c ± 8.75	5.18 c ± 0.25	0.96 b ± 0.48
45/30 °C	Control	55.16 d ± 2.8	83.10 d ± 4.54	50.17 d ± 0.78	170.12 c ± 11.20	8.00 c ± 0.41	2.04 a ± 0.14
	Potassium (1.5%)	87.41 b ± 5.1	258.73 b ± 13.65	100.62 b ± 1.5	328.44 a ± 22.10	14.25 b ± 0.70	0.96 c ± 0.051
	Zinc (0.2%)	111.83 a ± 6.2	310.77 a ± 16.31	104.79 a ± 1.3	337.90 a ± 20.30	15.23 a ± 0.84	0.87 d ± 0.044
	Boron (0.1%)	70.66 c ± 3.7	189.87 c ± 10.19	77.48 c ± 0.92	250.51 b ± 14.50	10.38 d ± 0.52	1.26 b ± 0.065
38/24 °C	Control	47.23 d ± 2.4	66.17 d ± 3.60	41.70 d ± 0.58	156.32 c ± 8.4	5.24 d ± 0.32	1.63 a ± 0.70
	Potassium (1.5%)	64.70 b ± 3.5	109.91 b ± 6.63	57.99 b ± 0.71	228.12 a ± 14.45	7.48 b ± 0.40	0.77 c ± 0.50
	Zinc (0.2%)	73.58 a ± 3.8	160.17 a ± 9.22	61.25 a ± 0.88	230.39 a ± 16.76	8.06 a ± 0.50	0.69 d ± 0.41
	Boron (0.1%)	51.29 c ± 2.8	85.16 c ± 4.62	48.71 c ± 0.64	205.57 b ± 11.20	6.21 c ± 0.35	1.08 b ± 0.64
	LSD	3.65	5.72	2.94	11.78	0.49	0.068

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 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 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 regimes and nutrients’ spray.

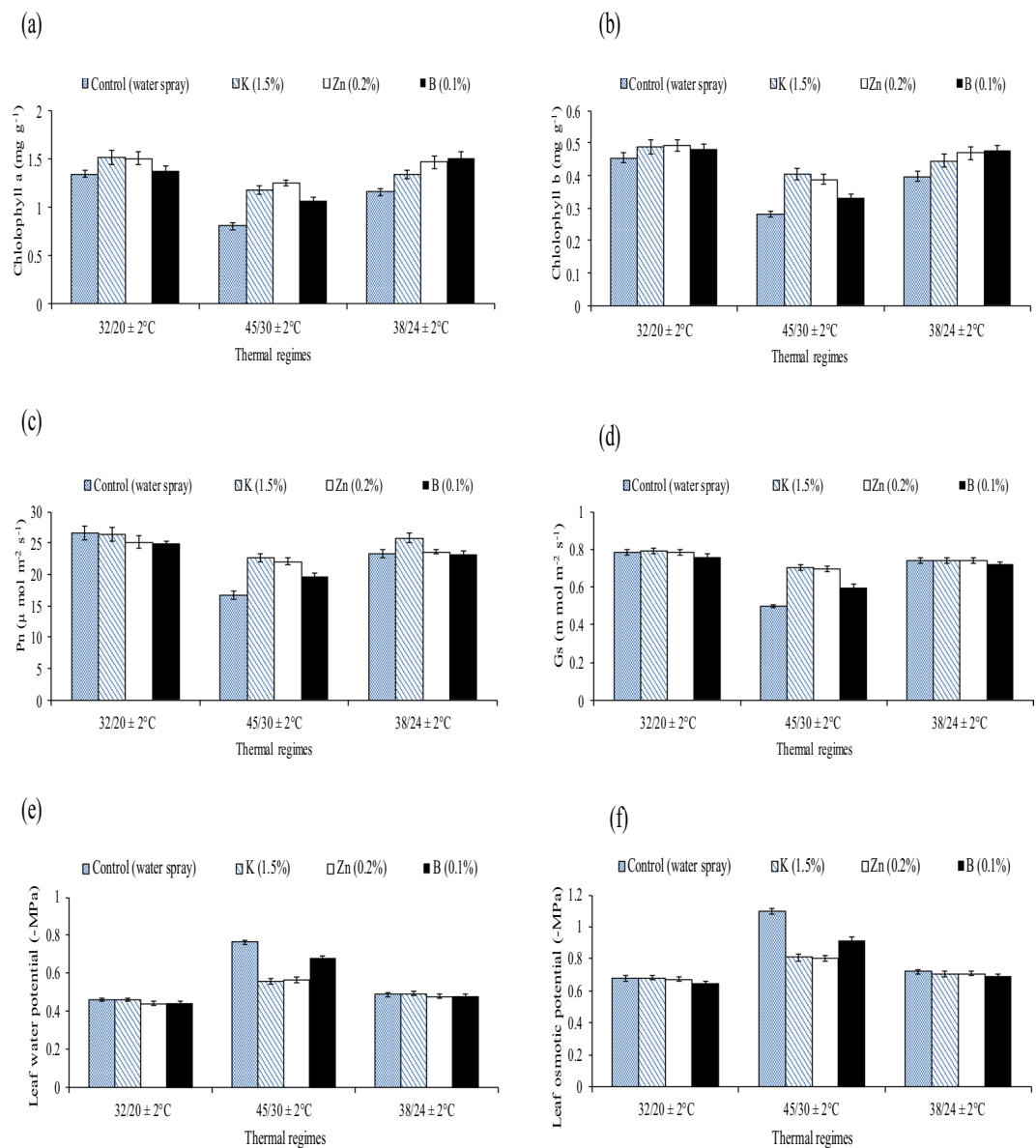


Figure 2. Effect of different thermal regimes and nutrients' spray on chlorophyll contents (a + b) (mg g⁻¹FW), net photosynthetic rate-Pn (μmol m⁻² sec⁻¹, FW), stomatal conductance (Gs m mol m⁻² s⁻¹), leaf water potential (-MPa) and leaf osmotic potential (-MPa) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions.

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). 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, UAF. 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

Thermal regimes	Nutrients	Chlorophyll a	Chlorophyll b	Pn	Gs	Water Potential	Osmotic potential
32/20 °C	Control	1.34 b ± 0.070	0.46 ab ± 0.27	26.69 a ± 0.63	0.79 a ± 0.042	0.46 a ± 0.041	0.68 a ± 0.034
	Potassium (1.5%)	1.51 a ± 0.073	0.48 a ± 0.30	26.38 a ± 0.67	0.79 a ± 0.040	0.46 a ± 0.040	0.68 a ± 0.032
	Zinc (0.2%)	1.50 a ± 0.076	0.49 a ± 0.32	26.20 a ± 0.57	0.78 a ± 0.035	0.44 a ± 0.039	0.67 a ± 0.035
	Boron (0.1%)	1.37 b ± 0.068	0.48 a ± 0.26	26.04 a ± 0.12	0.77 a ± 0.036	0.44 a ± 0.038	0.66 a ± 0.031
45/30 °C	Control	0.80 c ± 0.051	0.28 c ± 0.20	16.74 c ± 0.37	0.50 c ± 0.024	0.76 a ± 0.070	1.10 a ± 0.52
	Potassium (1.5%)	1.18 a ± 0.062	0.41 a ± 0.23	22.66 a ± 0.40	0.71 a ± 0.037	0.56 c ± 0.052	0.81 c ± 0.040
	Zinc (0.2%)	1.25 a ± 0.067	0.39 a ± 0.21	22.11 a ± 0.37	0.70 a ± 0.033	0.57 c ± 0.055	0.80 c ± 0.038
	Boron (0.1%)	1.07 b ± 0.055	0.33 b ± 0.19	19.79 b ± 0.29	0.60 b ± 0.031	0.68 b ± 0.064	0.91 b ± 0.045
38/24 °C	Control	1.16 c ± 0.059	0.40 c ± 0.22	22.29 b ± 0.35	0.74 a ± 0.034	0.49 a ± 0.044	0.72 a ± 0.030
	Potassium (1.5%)	1.33 b ± 0.063	0.45 ab ± 0.25	25.55 a ± 0.49	0.74 a ± 0.037	0.49 a ± 0.045	0.71 a ± 0.034
	Zinc (0.2%)	1.46 a ± 0.079	0.47 a ± 0.28	25.33 a ± 0.16	0.74 a ± 0.038	0.48 a ± 0.043	0.71 a ± 0.035
	Boron (0.1%)	1.50 a ± 0.086	0.48 a ± 0.31	23.87 b ± 0.37	0.72 a ± 0.033	0.48 a ± 0.046	0.70 a ± 0.035
	LSD	0.080	0.023	1.38	0.032	0.021	0.031

Table 3. Effect of different thermal regimes and nutrients' spray on chlorophyll contents (a + b) ($\text{mg g}^{-1}\text{FW}$), net photosynthetic rate-Pn ($\mu\text{mol m}^{-2} \text{sec}^{-1}$), FW), stomatal conductance (Gs $\text{m mol m}^{-2} \text{s}^{-1}$), leaf water potential ($-\text{MPa}$) and leaf osmotic potential ($-\text{MPa}$) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under glass house conditions. Values are the means of three replications ($n = 4$) \pm 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.

Thermal regimes	Nutrients	Boll Weight (g)	SCY
32/20 °C	Control	3.70 c ± 0.15	83.22 a ± 3.1
	Potassium (1.5%)	4.36 a ± 0.22	84.99 a ± 2.9
	Zinc (0.2%)	4.25 a ± 0.20	85.05 a ± 4.1
	Boron (0.1%)	4.13 ab ± 0.18	85.47 a ± 4.2
45/30 °C	Control	2.96 b ± 0.14	49.99 c ± 2.7
	Potassium (1.5%)	3.59 a ± 0.22	60.80 a ± 3.3
	Zinc (0.2%)	3.54 a ± 0.16	58.17 a ± 2.8
	Boron (0.1%)	3.54 a ± 0.17	53.67 b ± 2.7
38/24 °C	Control	3.38 b ± 0.11	67.69 c ± 4.4
	Potassium (1.5%)	3.70 a ± 0.19	77.45 a ± 4.0
	Zinc (0.2%)	3.75 a ± 0.15	75.92 a ± 3.8
	Boron (0.1%)	3.73 a ± 0.14	71.34 b ± 3.7
	LSD	0.20	2.99

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 ($n = 4$) \pm 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

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.

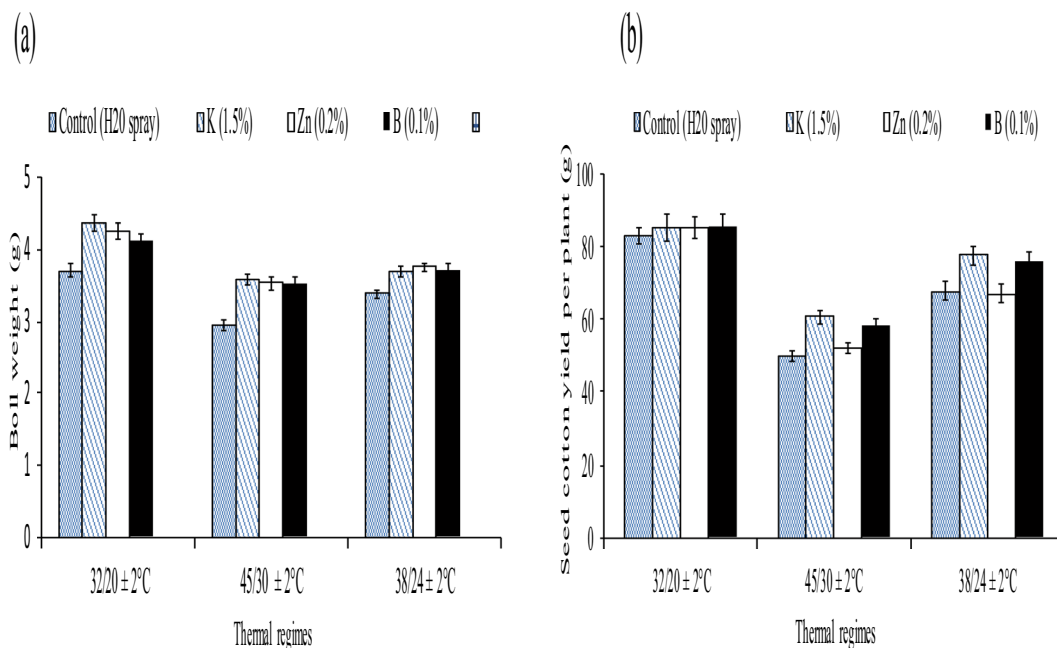


Figure 3. Effect of different thermal regimes and nutrients' spray on averaged boll weight (g) and seed cotton yield per plant (g) of cotton crop under glass house conditions.

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 ± 18.20
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	164.57 c ± 15.8	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 b ± 6.6	71.32 b ± 6.5	239.03 b ± 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 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.

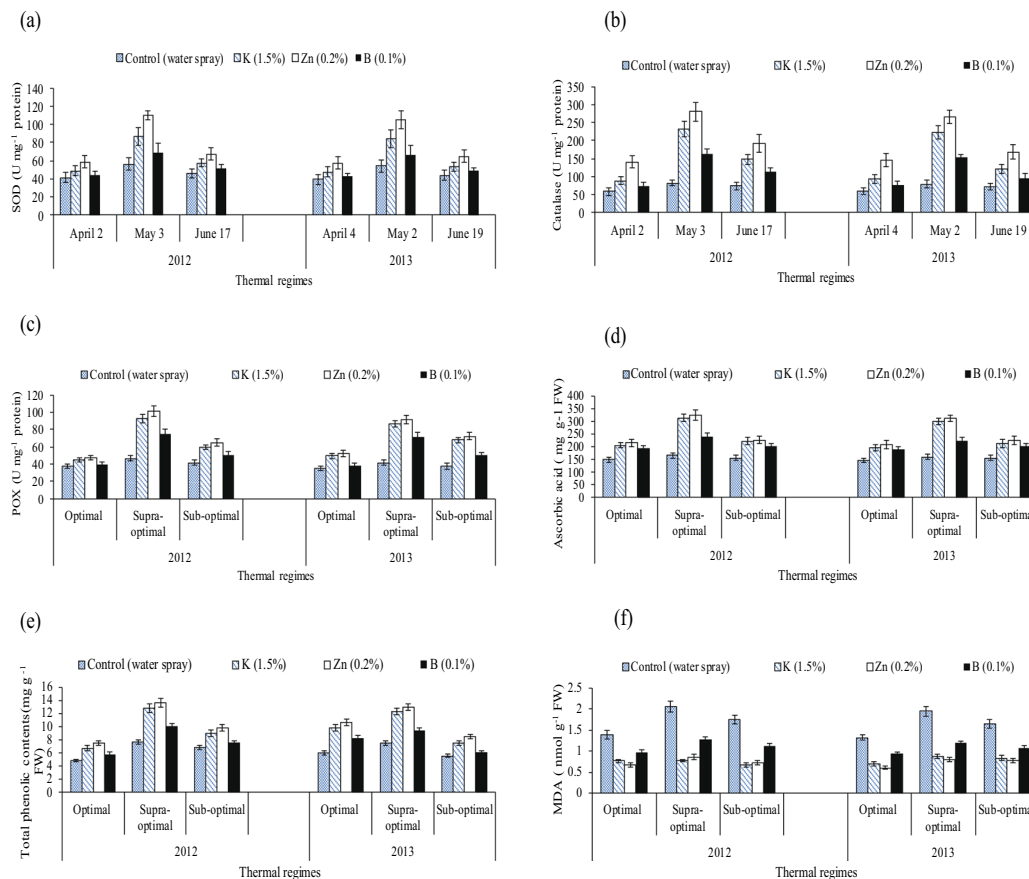


Figure 4. Effect of different thermal regimes and nutrients’ spray on effect of different thermal regimes and nutrients’ spray on superoxide dismutase (SOD), catalase (CAT), peroxidase (POX U mg⁻¹ protein), ascorbic acid (AsA mg g⁻¹ FW), total phenolic contents (TPC mg g⁻¹ FW) and malondialdehyde contents (MDA nmol g⁻¹ FW), (averaged across of squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013.

Thermal regimes	Nutrients	TPC 2012	TPC 2013	MDA 2012	MDA 2013	Chlorophyll a 2012	Chlorophyll a 2013	Chlorophyll b 2012	Chlorophyll b 2013
Optimal regimes of sowing dates	Control	4.86 b ± 0.37	5.98 c ± 0.45	1.39 a ± 0.87	1.32 a ± 0.97	1.48 ab ± 0.11	1.42 a ± 0.12	0.48 ab ± 0.39	0.50 a ± 0.42
	Potassium (1.5%)	6.40 a ± 0.52	9.82 a ± 0.80	0.76 c ± 0.065	0.70 c ± 0.62	1.62 a ± 0.13	1.50 a ± 0.11	0.52 a ± 0.45	0.54 a ± 0.50
	Zinc (0.2%)	7.40 a ± 0.60	10.60 a ± 0.91	0.66 c ± 0.052	0.60 c ± 0.48	1.56 a ± 0.10	1.52 a ± 0.13	0.53 a ± 0.47	0.54 a ± 0.48
	Boron (0.1%)	5.81 b ± 0.48	8.13 b ± 0.72	0.97 b ± 0.078	0.93 b ± 0.84	1.47 ab ± 0.12	1.38 a ± 0.09	0.52 a ± 0.50	0.50 a ± 0.35
Supra-optimal of sowing dates	Control	7.65 c ± 0.55	7.43 c ± 0.60	2.06 a ± 0.60	1.94 a ± 0.17	0.88 c ± 0.072	0.92 c ± 0.07	0.31 c ± 0.26	0.33 c ± 0.27
	Potassium (1.5%)	12.82 a ± 1.0	12.23 a ± 0.99	0.76 c ± 0.06	0.88 c ± 0.74	1.25 a ± 0.10	1.30 a ± 0.11	0.43 a ± 0.40	0.45 a ± 0.32
	Zinc (0.2%)	13.60 a ± 1.1	12.95 a ± 1.1	0.86 c ± 0.074	0.80 c ± 0.70	1.34 a ± 0.12	1.37 a ± 0.10	0.43 a ± 0.38	0.49 a ± 0.30
	Boron (0.1%)	9.97 b ± 0.80	9.29 b ± 0.80	1.28 b ± 0.94	1.19 b ± 0.10	1.18 ab ± 0.091	1.21 ab ± 0.08	0.36 b ± 0.30	0.39 b ± 0.25
Sub-optimal of sowing dates	Control	6.89 c ± 0.56	5.55 c ± 0.49	1.74 a ± 1.30	1.65 a ± 0.49	1.28 ab ± 0.11	1.26 b ± 0.09	0.42 bc ± 0.41	0.44 ab ± 0.36
	Potassium (1.5%)	8.93 a ± 0.75	7.53 a ± 0.62	0.67 c ± 0.62	0.83 c ± 0.77	1.37 a ± 0.10	1.41 a ± 0.11	0.47 a ± 0.45	0.50 a ± 0.39
	Zinc (0.2%)	9.80 a ± 0.85	8.42 a ± 0.72	0.72 c ± 0.68	0.76 c ± 0.66	1.41 a ± 0.13	1.47 a ± 0.13	0.47 a ± 0.44	0.48 a ± 0.41
	Boron (0.1%)	7.50 b ± 0.68	6.05 b ± 0.55	1.13 b ± 0.93	1.07 b ± 0.10	1.44 a ± 0.12	1.53 a ± 0.14	0.46 ab ± 0.30	0.48 a ± 0.38
	LSD	1.37	0.88	0.18	0.11	0.13	0.16	0.040	0.050

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.

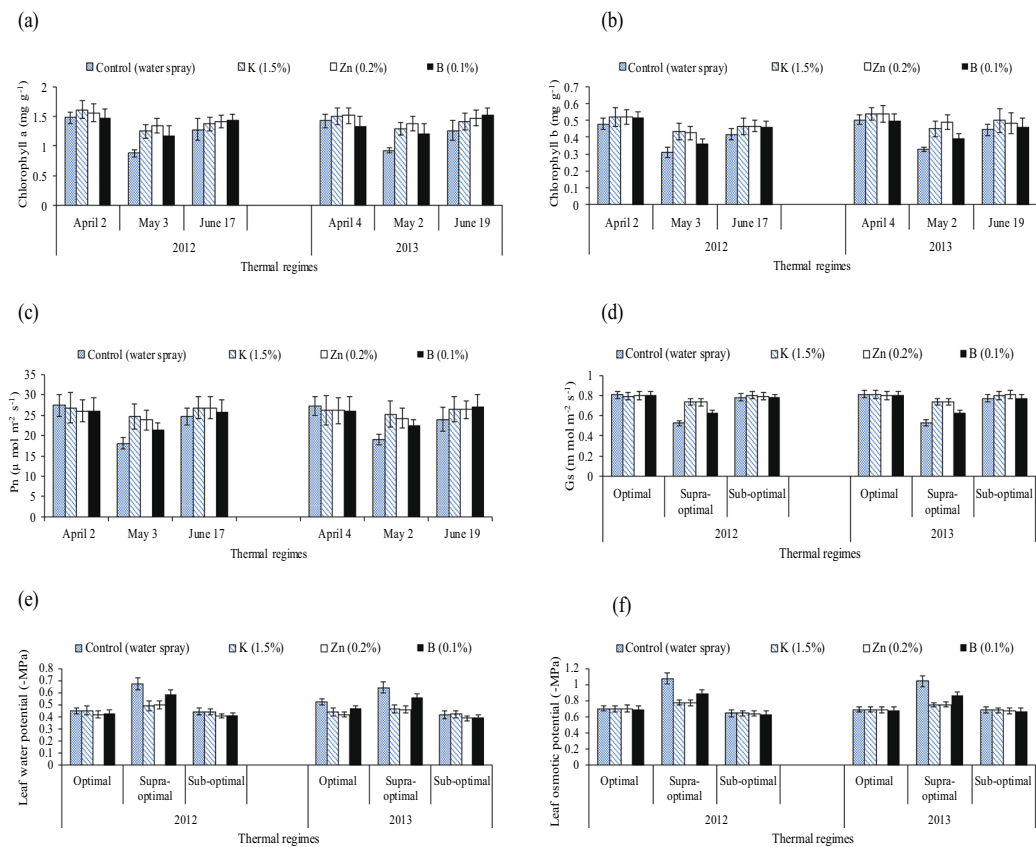


Figure 5. Effect of different thermal regimes and nutrients' spray on chlorophyll contents (a + b) ($\text{mg g}^{-1}\text{FW}$), net photosynthetic rate-Pn ($\mu\text{mol m}^{-2} \text{sec}^{-1}$, FW), stomatal conductance ($G_s \text{ m mol m}^{-2} \text{ s}^{-1}$), leaf water potential ($-\text{MPa}$) and leaf osmotic potential ($-\text{MPa}$) (averaged across of squaring, flowering and boll formation stages) of cotton leaves during 2012 and 2013.

Observations. 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 ²⁷. The samples for all enzymatic and non-enzymatic antioxidants were pipetted into 96-well plates. The plates were, then, read by micro plate reader (ELX800, Bio-Tek Instruments, Inc., Winooski, VT, USA) at different wavelengths. Superoxide dismutase contents were determined by²⁸ 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 H_2O_2 to H_2O and O_2 using the protocol as described by Liu²⁹. The reaction mixture [50 mM phosphate buffer (pH 7) + 5.9 mM H_2O_2] was mixed with 0.1 mL enzyme extract and read the absorbance at 240 nm. Peroxidase contents were determined using method as given by²⁹. Peroxidase was quantified as units of enzymes that oxidized guaiacol. The reaction mixture was comprised of 50 mM phosphate buffer (pH 5) + 40 mM H_2O_2 + 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 dist. H_2O + 100 μL sample extract + 1 mL dichlorophenol-indophenol + 100 μL 0.1% Meta H_3PO_4 were mixed in a test tube and absorbance was recorded at 520 nm³⁰. Folin-Ciocalteu (FC) reagent method was used for the determination of TPC³¹. 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 Na_2CO_3 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 by³². Leaf sample (0.5 g) was homogenized in 10 ml of 0.1% trichloroacetic acid (TCA) solution and centrifuged at $12000 \times g$ 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:

$$\text{MDA level (nmol)} = \frac{\Delta(A_{532 \text{ nm}} - A_{600 \text{ nm}})}{156 \times 10^5}$$

A = Absorption coefficient with the value of $156 \text{ mm}^{-1} \text{ cm}^{-1}$.

Thermal regimes	Nutrients	Pn 2012	Pn 2013	Gs 2012	Gs 2013	Water Potential 2012	Water Potential 2013	Osmotic Potential 2012	Osmotic Potential 2013
Optimal regimes of sowing dates	Control	27.43 a ± 2.4	27.27 a ± 2.5	0.81 a ± 0.076	0.81 a ± 0.075	0.45 a ± 0.039	0.46 a ± 0.041	0.70 a ± 0.065	0.69 a ± 0.062
	Potassium (1.5%)	26.83 a ± 2.1	26.18 a ± 2.2	0.79 a ± 0.073	0.81 a ± 0.077	0.45 a ± 0.035	0.44 a ± 0.038	0.70 a ± 0.062	0.69 a ± 0.065
	Zinc (0.2%)	26.05 a ± 2.3	26.07 a ± 1.9	0.80 a ± 0.071	0.80 a ± 0.075	0.42 a ± 0.040	0.42 a ± 0.036	0.70 a ± 0.069	0.68 a ± 0.059
	Boron (0.1%)	26.01 a ± 2.0	26.20 a ± 2.4	0.80 a ± 0.077	0.80 a ± 0.074	0.43 a ± 0.038	0.47 a ± 0.043	0.69 a ± 0.060	0.68 a ± 0.061
Supra-optimal of sowing dates	Control	18.06 c ± 1.6	18.92 c ± 1.3	0.52 c ± 0.049	0.53 c ± 0.049	0.67 a ± 0.057	0.64 a ± 0.059	1.07 a ± 0.091	1.04 a ± 0.091
	Potassium (1.5%)	24.62 a ± 1.9	25.20 a ± 1.8	0.73 a ± 0.068	0.74 a ± 0.069	0.49 c ± 0.042	0.46 c ± 0.044	0.78 c ± 0.072	0.75 c ± 0.070
	Zinc (0.2%)	23.76 a ± 1.8	24.26 a ± 2.1	0.73 a ± 0.069	0.73 a ± 0.071	0.50 c ± 0.046	0.46 c ± 0.041	0.78 c ± 0.068	0.75 c ± 0.068
	Boron (0.1%)	21.56 ab ± 1.6	22.10 ab ± 1.9	0.63 b ± 0.057	0.63 b ± 0.058	0.59 b ± 0.053	0.55 b ± 0.051	0.88 b ± 0.082	0.86 b ± 0.081
Sub-optimal of sowing dates	Control	24.58 ab ± 2.2	23.99 ab ± 2.2	0.78 a ± 0.073	0.77 a ± 0.071	0.44 a ± 0.038	0.42 a ± 0.037	0.65 a ± 0.057	0.68 a ± 0.061
	Potassium (1.5%)	26.83 a ± 2.5	26.56 a ± 2.5	0.80 a ± 0.076	0.80 a ± 0.075	0.44 a ± 0.041	0.42 a ± 0.038	0.64 a ± 0.059	0.68 a ± 0.058
	Zinc (0.2%)	27.80 a ± 2.6	26.41 a ± 2.3	0.79 a ± 0.074	0.81 a ± 0.079	0.41 a ± 0.038	0.39 a ± 0.033	0.64 a ± 0.062	0.68 a ± 0.063
	Boron (0.1%)	26.92 a ± 2.2	27.19 a ± 2.6	0.78 a ± 0.070	0.77 a ± 0.071	0.41 a ± 0.037	0.39 a ± 0.035	0.63 a ± 0.059	0.67 a ± 0.061
	LSD	2.98	3.01	0.096	0.089	0.08	0.073	0.10	0.11

Table 7. Effect of different thermal regimes and nutrients' spray on net photosynthetic rate-Pn ($\mu\text{mol m}^{-2} \text{sec}^{-1}$, FW), stomatal conductance ($G_s \text{ m mol m}^{-2} \text{s}^{-1}$), leaf water potential ($-\text{MPa}$) and leaf osmotic potential ($-\text{MPa}$) (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 ($n = 3$) \pm 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	Boll weight (g) 2012	Boll weight (g) 2013	SCY 2012	SCY 2013
April (High temperature) sub optimal	Control	3.53 a ± 0.31	3.64 b ± 0.32	87.60 b ± 8.4	85.60 b ± 8.1
	Potassium (1.5%)	4.13 a ± 0.38	4.24 a ± 0.39	101.34 a ± 9.9	99.34 a ± 9.4
	Zinc (0.2%)	4.05 a ± 0.40	4.16 a ± 0.37	98.44 a ± 9.2	97.62 a ± 9.2
	Boron (0.1%)	4.07 a ± 0.35	4.18 a ± 0.41	99.41 a ± 9.5	97.41 a ± 9.6
May (High temperature) supra	Control	2.88 b ± 0.23	2.99 b ± 0.26	70.11 b ± 6.3	68.45 b ± 6.4
	Potassium (1.5%)	3.41 a ± 0.29	3.52 a ± 0.32	82.89 a ± 7.7	81.22 a ± 7.8
	Zinc (0.2%)	3.36 a ± 0.31	3.46 a ± 0.29	82.37 a ± 7.9	81.37 a ± 7.5
	Boron (0.1%)	3.35 a ± 0.30	3.45 a ± 0.27	80.92 a ± 7.5	79.92 a ± 7.1
June (late sown as optimal)	Control	2.61 a ± 0.20	2.72 a ± 0.21	49.99 a ± 4.3	51.19 a ± 4.7
	Potassium (1.5%)	2.66 a ± 0.24	2.78 a ± 0.25	52.99 a ± 4.7	54.19 a ± 4.9
	Zinc (0.2%)	2.67 a ± 0.22	2.79 a ± 0.24	47.07 a ± 4.1	48.27 a ± 4.5
	Boron (0.1%)	2.61 a ± 0.18	2.73 a ± 0.26	47.33 a ± 4.0	48.53 a ± 4.3
	LSD	0.37	0.40	9.66	9.78

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 ($n = 3$) \pm 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.

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 method³³. 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:

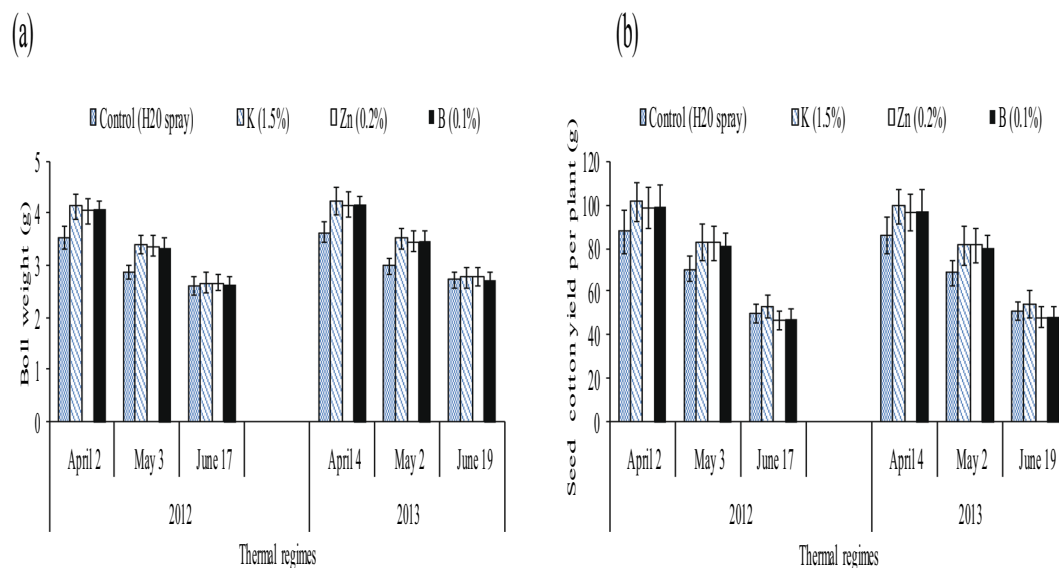


Figure 6. Effect of different thermal regimes and nutrients' spray on averaged boll weight (g) and seed cotton yield per plant (g) of cotton crop under field conditions during 2012 and 2013.

$$\text{Chl } a \left(\frac{\text{mg}}{\text{g FW}} \right) = [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] * \frac{V}{1000} * W$$

$$\text{Chl } b \left(\frac{\text{mg}}{\text{g FW}} \right) = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] * \frac{V}{1000} * W$$

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 (LCiAnalyser having Broad Head, Part Number LCi-002/B with Serial Number 32455). The P_n 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 described³⁴. Leaf water potential was determined through Scholander type pressure chamber (ARIMAD 2, Korea) following methodology as described by³⁵ 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 ($p \leq 0.05$). 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 \times main plots \times 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

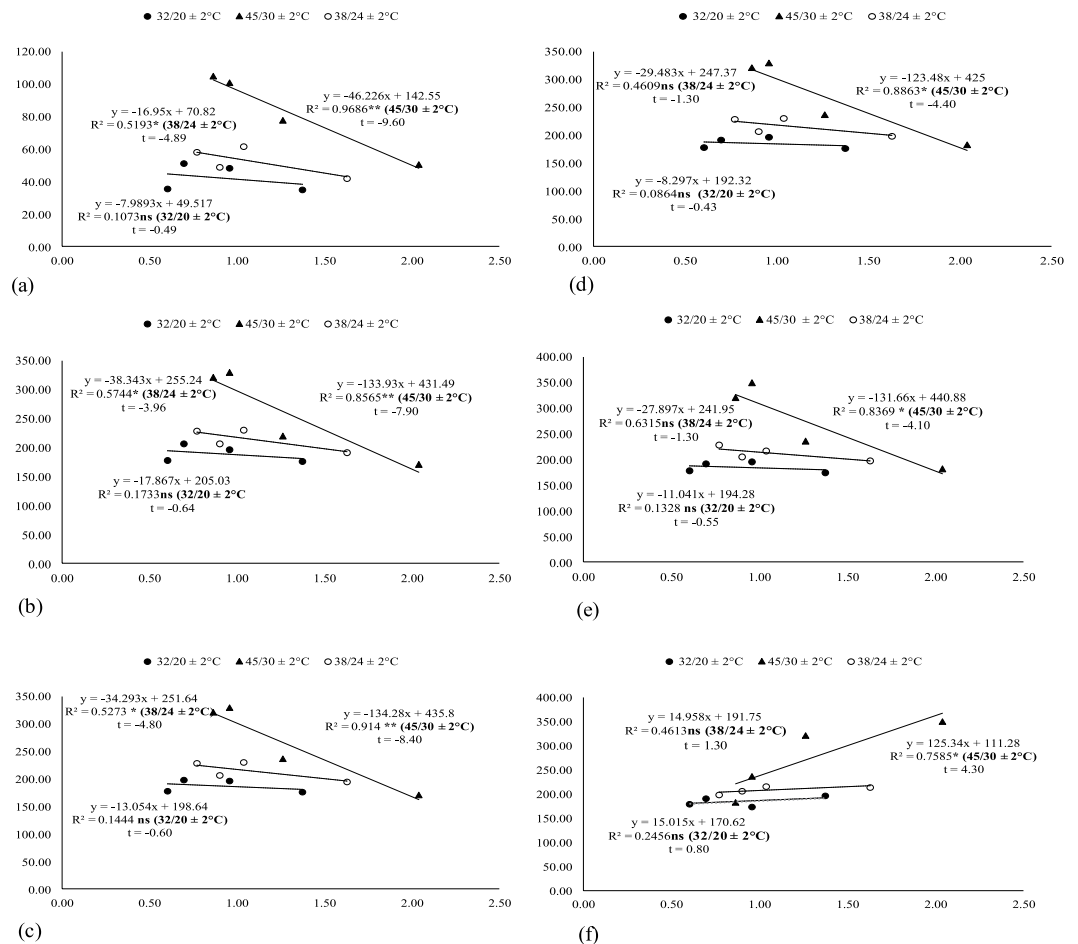


Figure 7. Association between malondialdehyde with (a) peroxidase, (b) ascorbic acid and (c) total phenolic contents, (d) stomatal conductance, (e) boll weight and (f) of stomatal conductance with water potential under glass house conditions (averaged across of three reproductive stages). * and ** indicates significance at 5 and 1% levels, respectively.

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 with '0.2% Zn' compared to water spray. Likewise, Zn also enhanced the POX, AsA and TPC contents under optimal, sub optimal and supra optimal temperature regimes. Moreover, MDA synthesis was downregulated by 56%, 58% and 57% with '0.2% Zn' compared to 'water treated plants' 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.

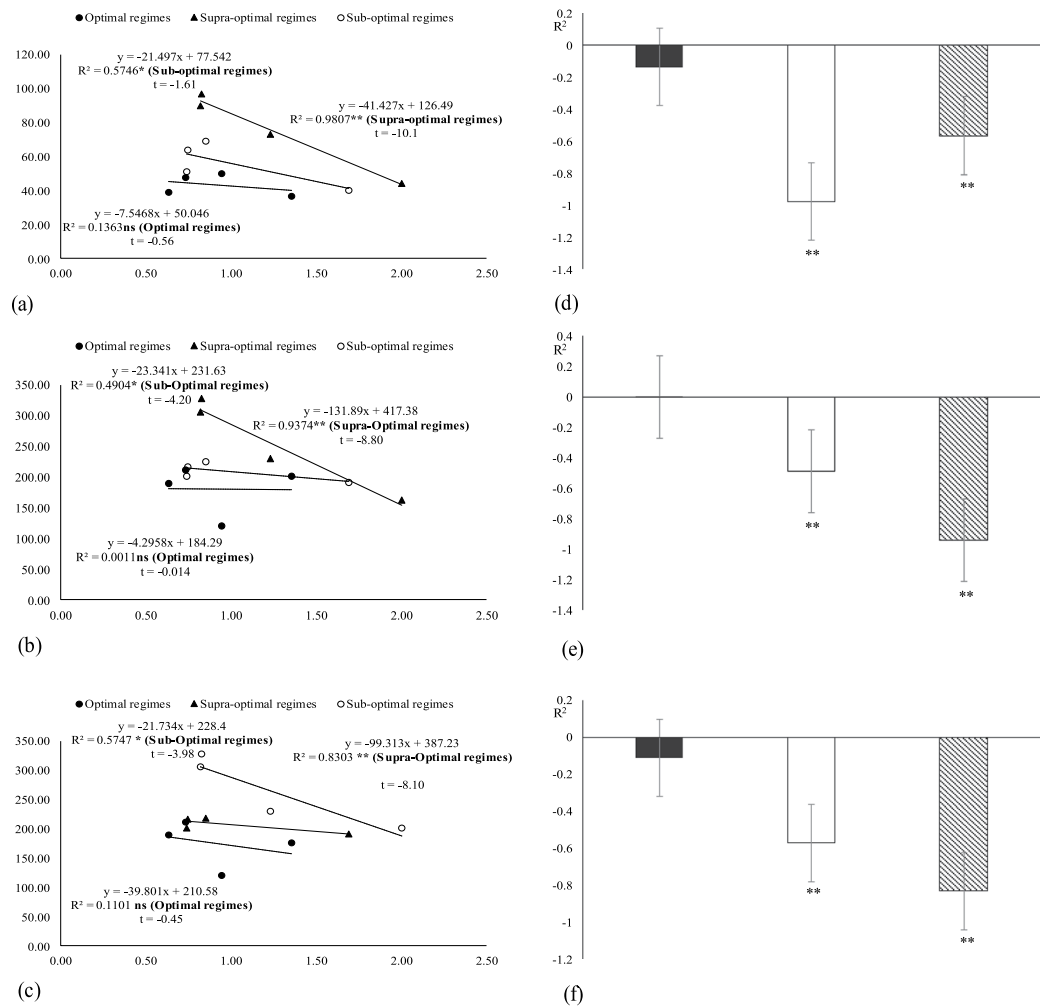


Figure 8. Association between malondialdehyde with (a) peroxidase, (b) ascorbic acid and (c) total phenolic contents under field conditions (averaged across of three reproductive stages). * and ** indicates significance at 5 and 1% levels, respectively.

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

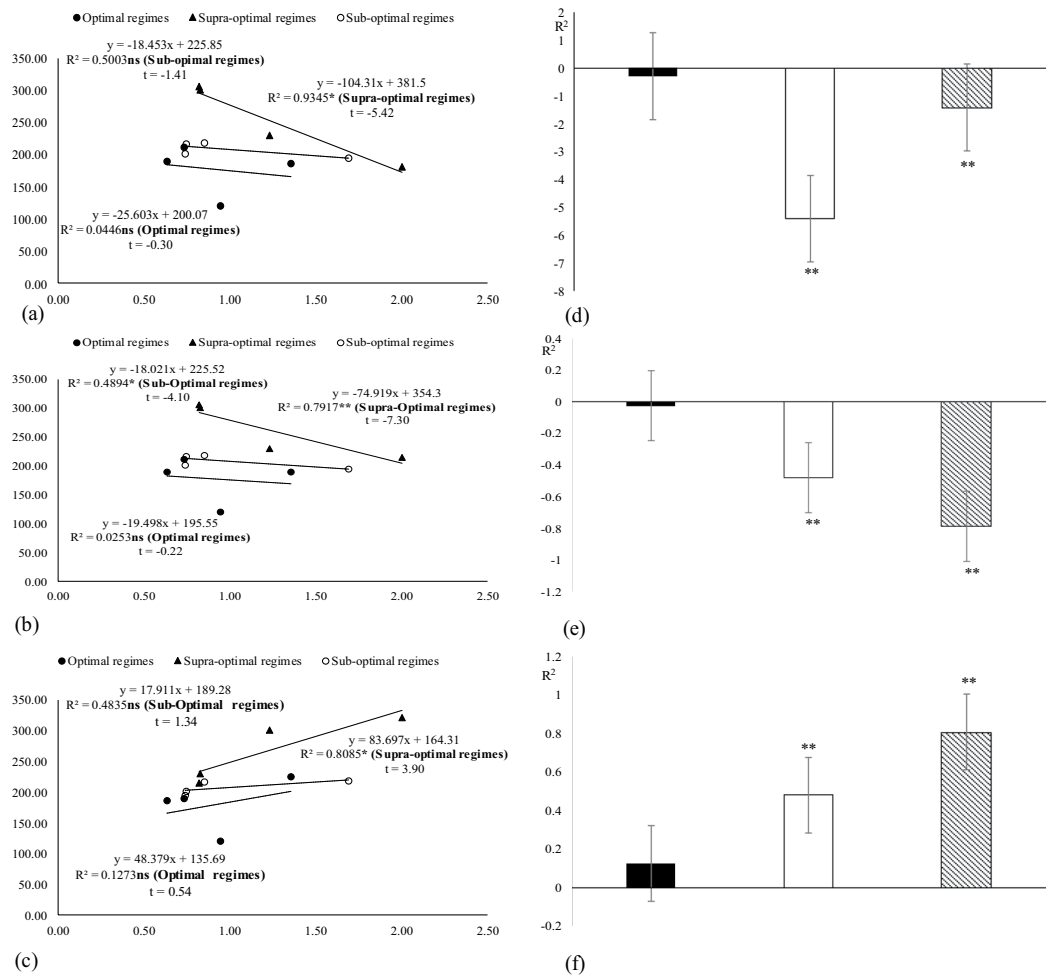


Figure 9. Association between malondialdehyde with (a) stomatal conductance, (b) boll weight and (c) stomatal conductance with water potential under field conditions (averaged across of three reproductive stages). * and ** indicates significance at 5 and 1% levels, respectively.

Correlation components	CAT	Chlor a	Chlor b	Pn	SCY
Chlor a	0.47*				
Chlor b	0.48*	0.96**			
Pn	0.51*	0.98**	0.98**		
SCY	0.89**	0.71**	0.69**	0.68**	
SOD	0.76**	0.85**	0.82**	0.85**	0.67**

Table 9. Correlation between Chlor.a/b, Pn, CAT, SOD and SCY under glass house conditions. 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 level. n (number of pairs of observations) = 48.

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 across during both years of study and of three reproductive stages. Significantly more net photosynthetic rate, stomatal conductance, water potential and less osmotic potential were

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	0.69**	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*8	0.69**

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.

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

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).

Regression and correlation of studied components under both glasshouse and field conditions during 2012 and 2013.

The regression analysis under glasshouse conditions indicated that the malondialdehyde has strong negative relation with POX, AsA, TPC and with stomatal conductance. While water relations have positive relation with stomatal conductance (Fig. 7). The regression analysis under field conditions indicated that the malondialdehyde has strong negative relation with POX, AsA, TPC and with stomatal conductance while water relations have strong positive relation with stomatal conductance (Figs 8 and 9).

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 relation 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 yield¹⁴. Reactive oxygen species (ROS) affected the membranes of each organelle for example the integrity of chloroplast/photosynthetic machinery^{36,37}. A balance is required for ROS and antioxidants for the normal functions of plant defensive system. The oxidative stress causes the blockage of nutrients channels^{5,38}. The high temperature regimes (45/30 °C) of glass house and (April and May) of field study upregulated the superoxide dismutase, catalase, peroxidase, ascorbic acid and total phenolic contents. However, cotton plants were unable to protect the cells from MDA contents due to oxidative stress⁶ indicating that the optimum temperature for biochemical and metabolic functions ranges between 25 to 31 °C³⁹. In this study, the high temperature stress reduced chlorophyll contents, net photosynthetic rate and stomatal conductance^{40,41} indicating that photosynthetic apparatus is most sensitive to heat stress⁴². It may be the outcome of breakdown of photosynthetic pigments, associated proteins⁴³, reduction in membrane integrity⁴⁴, inefficiency of PS-II due to disruption of thylakoid structure⁴⁵ and of linked enzymes⁴⁶. The reduction in stomatal conductance under high temperature stress⁴⁷ might be due to the stress signals from roots and consequent production of ethylene⁴⁸ (Tables 3 and 7, Figs 2 and 5). In this study, leaf water potential was decreased while osmotic potential was increased under high temperature stress, as reported by¹¹. It might be due to roots inability to up take water and nutrients under high temperature^{49,50}. High temperature regimes of glass house and high temperature regimes (April and May thermal regimes) of field study showed reduction in averaged boll weight and seed cotton yield per plant as reported by¹⁴. This may be due to the production of stress hormones^{51,52} which may decrease the production and translocation of photo assimilates for developing bolls^{53,54}.

The foliar spray of K, Zn and Boron activated the plant defensive system and reduced the MDA contents from medium to high temperature regimes (Tables 2 and 6; Figs 1 and 5) as reported by^{21,55,56}. This may be due to the reduction in oxidative stress and the stress hormones^{24,57}.

In this study, foliar application of K, Zn and B improved the light harvesting pigments (chlorophyll *a* and *b* contents)^{58–60} (Tables 3 and 6, Figs 2 and 5). This may be due to the alleviation of adverse impacts of oxidative stress that may boost the chlorophyll fluorescence and the energy output of light reactions^{61,62}.

In this study, both potassium and zinc enhanced net photosynthetic rate and stomatal conductance^{63,64} might be due to the role of these nutrients in CO₂ assimilation and in photosynthetic process^{65–67}.

Foliar spray of potassium, zinc and boron increased water potential and reduced solute potential^{68–70} as found in this study which might be due to less relative cell injury of membranes^{21,71,72}.

In this study, foliar spray of potassium, and zinc upsurged the boll weight and seed cotton yield even in K and Zn enriched soil^{73,74}. This may be the outcome of the higher production of carbohydrates⁷⁵. 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 by⁶.

Conclusion

High temperature stress at three reproductive stages of cotton crop caused yield reduction which was 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.

References

- Dinar, A., Hassan, R., Mendelsohn, R. & Benhin, J. *Climate change and agriculture in Africa: impact assessment and adaptation strategies* (2012).
- Pachauri, R. K. *et al. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change* (2014).
- Gür, A., Demirel, U., Özden, M., Kahraman, A. & Çopur, O. Diurnal gradual heat stress affects antioxidant enzymes, proline accumulation and some physiological components in cotton (*Gossypium hirsutum* L.). *African Journal of Biotechnology* **9**, 1008–1015 (2010).
- Sarwar, M. *et al.* Hydrogen peroxide reduces heat-induced yield losses in cotton (*Gossypium hirsutum* L.) by protecting cellular membrane damage. *Journal of Agronomy and Crop Science* (2017).
- Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R. & Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International journal of molecular sciences* **14**, 9643–9684 (2013).
- Sarwar, M. *et al.* Exogenously applied growth regulators protect the cotton crop from heat-induced injury by modulating plant defense mechanism. *Scientific reports* **8**, 17086 (2018).
- Shahid, M., Saleem, M. F., Anjum, S. A., Shahid, M. & Afzal, I. Effect of terminal heat stress on proline, secondary metabolites and yield components of wheat (*Triticum aestivum* L.) genotypes. *The Philippine Agricultural Scientist* **100**, 278–286 (2017).
- Pettigrew, W. Cultivar variation in cotton photosynthetic performance under different temperature regimes. *Photosynthetica*, 1–6 (2016).
- Dabbert, T. & Gore, M. A. Challenges and perspectives on improving heat and drought stress resilience in cotton. *Journal of Cotton Science* **18**, 393–409 (2014).
- Conaty, W., Burke, J., Mahan, J., Neilsen, J. & Sutton, B. Determining the optimum plant temperature of cotton physiology and yield to improve plant-based irrigation scheduling. *Crop Science* **52**, 1828–1836 (2012).
- Wahid, A. & Close, T. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum* **51**, 104–109 (2007).
- Fahad, S. *et al.* Exogenously applied plant growth regulators enhance the morpho-physiological growth and yield of rice under high temperature. *Frontiers in Plant Science* **7** (2016).
- Lokhande, S. & Reddy, K. R. Quantifying temperature effects on cotton reproductive efficiency and fiber quality. *Agronomy Journal* **106**, 1275–1282 (2014).
- Singh, R. P., Prasad, P. V., Sunita, K., Giri, S. & Reddy, K. R. Influence of high temperature and breeding for heat tolerance in cotton: a review. *Advances in Agronomy* **93**, 313–385 (2007).
- Awasthi, R. *et al.* Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. *Functional Plant Biology* **41**, 1148–1167 (2014).
- Waraich, E., Ahmad, R., Halim, A. & Aziz, T. Alleviation of temperature stress by nutrient management in crop plants: a review. *Journal of soil science and plant nutrition* **12**, 221–244 (2012).
- Ahmad, P. & Prasad, M. N. V. *Abiotic stress responses in plants: metabolism, productivity and sustainability*. (Springer Science & Business Media, 2011).
- Adiloglu, A. & Adiloglu, S. The effect of boron (B) application on the growth and nutrient contents of maize in zinc (Zn) deficient soils. *Research Journal of Agriculture and Biological Sciences* **2**, 1–4 (2006).
- Dordas, C. Foliar boron application affects lint and seed yield and improves seed quality of cotton grown on calcareous soils. *Nutrient Cycling in Agroecosystems* **76**, 19–28 (2006).
- Oosterhuis, D. M. Physiological aspects of potassium deficiency in cotton. *Special Reports-University Of Arkansas Agricultural Experiment Station* **183**, 61–73 (1997).
- Corrales, I., Poschenrieder, C. & Barceló, J. Boron-induced amelioration of aluminium toxicity in a monocot and a dicot species. *Journal of plant physiology* **165**, 504–513 (2008).
- Upadhyaya, H., Dutta, B. K. & Panda, S. K. Zinc modulates drought-induced biochemical damages in tea [*Camellia sinensis* (L.) O Kuntze]. *Journal of agricultural and food chemistry* **61**, 6660–6670 (2013).
- Zahoor, R. *et al.* Potassium improves photosynthetic tolerance to and recovery from episodic drought stress in functional leaves of cotton (*Gossypium hirsutum* L.). *Plant physiology and biochemistry* **119**, 21–32 (2017).
- Marschner, H. Marschner's mineral nutrition of higher plants. *Academic Press* Vol. 89 (2012).
- Chen, W., Yang, X., He, Z., Feng, Y. & Hu, F. Differential changes in photosynthetic capacity, 77 K chlorophyll fluorescence and chloroplast ultrastructure between Zn-efficient and Zn-inefficient rice genotypes (*Oryza sativa*) under low zinc stress. *Physiologia plantarum* **132**, 89–101 (2008).
- Stavrianakou, S., Liakopoulos, G. & Karabourniotis, G. Boron deficiency effects on growth, photosynthesis and relative concentrations of phenolics of *Dittrichia viscosa* (Asteraceae). *Environmental and experimental botany* **56**, 293–300 (2006).
- Terras, F. R. *et al.* A new family of basic cysteine-rich plant antifungal proteins from Brassicaceae species. *FEBS letters* **316**, 233–240 (1993).
- Giannopolitis, C. N. & Ries, S. K. Superoxide dismutases I. Occurrence in higher plants. *Plant physiology* **59**, 309–314 (1977).
- Liu, D., Zou, J., Meng, Q., Zou, J. & Jiang, W. Uptake and accumulation and oxidative stress in garlic (*Allium sativum* L.) under lead phytotoxicity. *Ecotoxicology* **18**, 134–143 (2009).
- Al-Yousef, S. A. Antifungal Activity of Volatiles from Lemongrass (*Cymbopogon citratus*) and Peppermint (*Mentha piperita*) Oils Against Some Respiratory Pathogenic Species of *Aspergillus*. *Int. J. Curr. Microbiol. App. Sci* **2**, 261–272 (2013).
- Ainsworth, E. A. & Gillespie, K. M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols* **2**, 875–877 (2007).

32. Cakmak, I. & Horst, W. J. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* **83**, 463–468 (1991).
33. Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology* **24**, 1 (1949).
34. Silveira, J. A. G., Araújo, S. A. M., Lima, J. P. M. S. & Viégas, R. A. Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*. *Environmental and Experimental Botany* **66**, 1–8 (2009).
35. Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. & Hammel, H. Sap pressure in vascular plants negative hydrostatic pressure can be measured in plants. *Science* **148**, 339–346 (1965).
36. Mueller, M. J. & Berger, S. Reactive electrophilic oxylipins: pattern recognition and signalling. *Phytochemistry* **70**, 1511–1521 (2009).
37. Waszczak, C., Carmody, M. & Kangasjärvi, J. Reactive oxygen species in plant signaling. *Annual review of plant biology* **69**, 209–236 (2018).
38. Krieger-Liszczay, A. Singlet oxygen production in photosynthesis. *Journal of experimental botany* **56**, 337–346 (2005).
39. Burke, J. J. & Wanjura, D. F. In *Physiology of cotton* 123–128 (Springer, 2010).
40. Allakhverdiev, S. I. *et al.* Heat stress: an overview of molecular responses in photosynthesis. *Photosynthesis research* **98**, 541 (2008).
41. Hemantaranjan, A. *et al.* Heat stress responses and thermotolerance. *Adv. Plants Agric. Res* **1**(10), 15406 (2014).
42. Loka, D. A. & Oosterhuis, D. M. Effect of high night temperatures during anthesis on cotton (*Gossypium hirsutum* L.) pistil and leaf physiology and biochemistry. *Australian Journal of Crop Science* **10**, 741 (2016).
43. Kreslavski, V., Tatarinzev, N., Shabnova, N., Semenova, G. & Kosobryukhov, A. Characterization of the nature of photosynthetic recovery of wheat seedlings from short-term dark heat exposures and analysis of the mode of acclimation to different light intensities. *Journal of plant physiology* **165**, 1592–1600 (2008).
44. Howarth, C. Genetic improvements of tolerance to high temperature. *Abiotic stresses: plant resistance through breeding and molecular approaches*. Howarth Press Inc., New York (2005).
45. Zhang, X., Hegerl, G., Zwiers, F. W. & Kenyon, J. Avoiding inhomogeneity in percentile-based indices of temperature extremes. *Journal of Climate* **18**, 1641–1651 (2005).
46. Mathur, S. & Jajoo, A. In *Physiological mechanisms and adaptation strategies in plants under changing environment* 163–191 (Springer, 2014).
47. Carmo-Silva, A. E. *et al.* Decreased CO₂ availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. *Environmental and Experimental Botany* **83**, 1–11 (2012).
48. Jiroutova, P., Oklestkova, J. & Strnad, M. Crosstalk between brassinosteroids and ethylene during plant growth and under abiotic stress conditions. *International journal of molecular sciences* **19**, 3283 (2018).
49. Ghorbanpour, A., Salimi, A., Ghanbary, M. A. T., Pirdashti, H. & Dehestani, A. The effect of *Trichoderma harzianum* in mitigating low temperature stress in tomato (*Solanum lycopersicum* L.) plants. *Scientia Horticulturae* **230**, 134–141 (2018).
50. Pei, Z.-M. *et al.* Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406**, 731–734 (2000).
51. Larkindale, J. & Huang, B. Thermotolerance and antioxidant systems in *Agrostis stolonifera*: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. *Journal of plant physiology* **161**, 405–413 (2004).
52. Ullah, A., Sun, H., Yang, X. & Zhang, X. Drought coping strategies in cotton: increased crop per drop. *Plant biotechnology journal* **15**, 271–284 (2017).
53. Sekmen, A. H., Ozgur, R., Uzilday, B. & Turkan, I. Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum*) cultivars under combined drought and heat induced oxidative stress. *Environmental and Experimental Botany* **99**, 141–149 (2014).
54. Snider, J. L., Oosterhuis, D. M., Loka, D. A. & Kawakami, E. M. High temperature limits *in vivo* pollen tube growth rates by altering diurnal carbohydrate balance in field-grown *Gossypium hirsutum* pistils. *Journal of plant physiology* **168**, 1168–1175 (2011).
55. Han, S. *et al.* Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. *Journal of plant physiology* **165**, 1331–1341 (2008).
56. Lester, G. E., Jifon, J. L. & Makus, D. J. Impact of potassium nutrition on postharvest fruit quality: Melon (*Cucumis melo* L.) case study. *Plant and soil* **335**, 117–131 (2010).
57. Disante, K. B., Fuentes, D. & Cortina, J. Response to drought of Zn-stressed *Quercus suber* L. seedlings. *Environmental and experimental botany* **70**, 96–103 (2011).
58. Hu, W., Coomer, T. D., Loka, D. A., Oosterhuis, D. M. & Zhou, Z. Potassium deficiency affects the carbon-nitrogen balance in cotton leaves. *Plant physiology and biochemistry* **115**, 408–417 (2017).
59. Ibrahim, E. A. & Ramadan, W. A. Effect of zinc foliar spray alone and combined with humic acid or/and chitosan on growth, nutrient elements content and yield of dry bean (*Phaseolus vulgaris* L.) plants sown at different dates. *Scientia Horticulturae* **184**, 101–105 (2015).
60. Seth, K. & Aery, N. C. Effect of boron on the contents of chlorophyll, carotenoid, phenol and soluble leaf protein in mung bean, *Vigna radiata* (L.) Wilczek. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **84**, 713–719 (2014).
61. Eisvand, H., Kamaei, H. & Nazarian, F. Chlorophyll fluorescence, yield and yield components of bread wheat affected by phosphate bio-fertilizer, zinc and boron under late-season heat stress. *Photosynthetica* **56**, 1287–1296 (2018).
62. Singh, S. K. & Reddy, V. R. Potassium starvation limits soybean growth more than the photosynthetic processes across CO₂ levels. *Frontiers in plant science* **8**, 991 (2017).
63. Hu, W. *et al.* Potassium (K) supply affects K accumulation and photosynthetic physiology in two cotton (*Gossypium hirsutum* L.) cultivars with different K sensitivities. *Field Crops Research* **196**, 51–63 (2016).
64. Wang, H. & Jin, J. Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. *Photosynthetica* **43**, 591–596 (2005).
65. Tsonev, T. & Cebola Lidon, F. J. Zinc in plants-An overview. *Emirates Journal of Food & Agriculture (EJFA)* **24** (2012).
66. Hu, W. *et al.* Potassium application affects carbohydrate metabolism in the leaf subtending the cotton (*Gossypium hirsutum* L.) boll and its relationship with boll biomass. *Field Crops Research* **179**, 120–131 (2015).
67. Tsialtas, I. T., Shabala, S., Baxevanos, D. & Matsi, T. Effect of potassium fertilization on leaf physiology, fiber yield and quality in cotton (*Gossypium hirsutum* L.) under irrigated Mediterranean conditions. *Field crops research* **193**, 94–103 (2016).
68. Karim, M. R. *et al.* Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. *Journal of Plant Nutrition and Soil Science* **175**, 142–151 (2012).
69. Pervez, H., Ashraf, M. & Makhdum, M. Influence of potassium nutrition on gas exchange characteristics and water relations in cotton (*Gossypium hirsutum* L.). *Photosynthetica* **42**, 251–255 (2004).
70. Sadoogh, F., Shariatmadari, H., Khoshgoftarmanesh, A. & Mosaddeghi, M. Adjusted nutrition of tomato with potassium and zinc in drought stress conditions induced by polyethylene glycol 6000 in hydroponic culture. *Journal of Science and Technology of Greenhouse Culture* **5** (2014).
71. Ahanger, M. A., Agarwal, R., Tomar, N. S. & Shrivastava, M. Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L. cultivar Kent). *Journal of plant interactions* **10**, 211–223 (2015).
72. Rehman, H.-U., Aziz, T., Farooq, M., Wakeel, A. & Rengel, Z. Zinc nutrition in rice production systems: a review. *Plant and soil* **361**, 203–226 (2012).
73. Oosterhuis, D. M., Loka, D. A. & Raper, T. B. Potassium and stress alleviation: Physiological functions and management of cotton. *Journal of Plant Nutrition and Soil Science* **176**, 331–343 (2013).

74. Soomro, A. *et al.* Effect of boron and zinc micronutrients on seed cotton yield and its components. *Pak. J. Biol. Sci* **2**, 1507–1509 (2000).
75. Rashidi, M. & Seilsepour, M. Response of yield, yield components and fiber properties of cotton to different application rates of nitrogen and boron. *Journal of Environmental Science and Engineering* **5**, 1147–1154 (2011).

Acknowledgements

The authors are highly thankful to the University of Agriculture Faisalabad, Pakistan; the Analytical Lab, Department of Agronomy, University of Agriculture Faisalabad, Pakistan, for providing technical support and glasshouse/laboratory facilities. The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding Research group No. (RGP-199).

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 Alyemini, Parvaiz Ahmad, provided the technical support and chemicals required for the experiment.

Additional Information

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019