

EFFECTS OF ASCORBIC ACID ON MEMBRANE STABILITY AND YIELD OF HEAT-STRESSED BT COTTON

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ABSTRACT

Among all the environmental factors heat stress has a key role in growth and yield reduction of cotton crop (*Gossypium hirsutum* L.). The present study was carried out to look into the effects of foliar applied ascorbic acid on cotton crop grown under heat stress condition. Experiment was planted during 2013 and repeated during 2014 at the Agronomic Research Area, University of Agriculture Faisalabad, Pakistan. Experimental material was comprised of heat stress imposition (H₀= No heat imposition, H₁= Heat imposition at square initiation, H₂= Heat imposition at flower initiation) and levels of foliar applied ascorbic acid (A₀= control/water spray, A₁= 20 mg L⁻¹, A₂= 40 mg L⁻¹ and A₃= 60 mg L⁻¹). Heat was imposed to cotton crop by covering the plots with polythene sheet at reproductive stages for 5 days. Results showed that heat stress badly affected the yield and related components of cotton crop. Cell membrane thermostability (CMT) was also reduced under high temperature stress. Foliar application of ascorbic acid at 40 mg L⁻¹ reduced the cell injury and improved yield and related components under all heat treatments.

Key words: *Gossypium hirsutum*; heat tolerance, sympodial branches, boll weight, boll number

INTRODUCTION

The global temperature will rise at 0.6°C till the 19th century and is expected to increase by 1.4 to 5.8°C by the end of the century (Houghton *et al.*, 2001). Heat stress can be defined as an increase in temperature beyond a threshold level for a duration which may cause permanent damage to plant growth and development (Wahid *et al.*, 2007).

Cotton (*Gossypium hirsutum* L.) is one of the important cash crops of world. High temperature during the growing season of cotton is one of the main factors reducing its productivity (Rana *et al.*, 2011). Among all the abiotic factors which are responsible for reduction in yield, high temperature is the major one. All growth stages of cotton crop were adversely affected by high temperatures but, reproduction is the most sensitive in this regard (Oosterhuis, 2002). The daily standard temperature for best growth of cotton crop is 27 to 29°C (Reddy *et al.*, 2004). Above 32°C the reproductive efficiency of the cotton crop is badly affected in different ways, e.g., inhibition of photosynthesis and crop growth rate, decreased metabolism, pollination and fertilization (Snider *et al.*, 2009). A temperature of about 33°C was found optimum for photosynthesis efficiency of cotton and a significant decrease in carbon fixation at 36°C (Bibi *et al.*, 2008). Cellular membranes are highly sensitive to abiotic stresses (Tayefi *et al.*, 2011). Temperatures above 35°C increased membrane leakage and decreased leaf extension in cotton (Bibi *et al.*, 2008). High temperature leads to the production of reactive oxygen species (ROS)

such as H₂O₂, hydroxyl ions, singlet oxygen etc. (Foyer *et al.*, 2009). Hydrogen peroxide has long life damaging the membranes of all organelles of the cells (Adachi *et al.*, 2009). So, improving heat tolerance of plant is a viable approach to resolve this problem induced by global warming. It is necessary to find out the responses of plants to heat stress and their fundamental physiological mechanisms, as it can provide insights into how plants may be modified to develop into more tolerant (Wang *et al.*, 2010).

Ascorbate is a major metabolite in plants performing a key role in plant protection against numerous environmental stresses like high temperature, salinity etc. (Vwioko *et al.*, 2008). Ascorbic acid is used as an indication compound in many studies which are related with alleviation of stress (Lopez *et al.*, 2011). Ascorbic acid is regarded as one of the most effective growth regulators in different abiotic stresses (Conklin, 2001) playing multiple roles in many developmental processes (Pignocchi and Foyer, 2003). Ascorbic acid, being an antioxidant, actively scavenges reactive oxygen species (ROS), reducing the chlorophyll degradation under salinity (Ashraf, 2009). It has ability to reduce the adverse impacts of stress on plants by neutralizing harmful oxidants which damage plant membranes such as thylakoid membranes of chloroplasts (Dolatabadian *et al.*, 2009).

Keeping in view above review, present study was planned to determine the effect of foliar applied ascorbic acid on cell membrane thermostability and yield of heat-stressed Bt cotton.

MATERIALS AND METHODS

The field experiment was conducted for two consecutive years i.e. 2013 and 2014 at the Research Area, Department of Agronomy, University of Agriculture Faisalabad, Pakistan. The delinted seed of Bt cotton (cv. MNH-886) at the rate of 20 kg ha⁻¹, was dibbled at 30 cm distance on one side of 75 cm apart ridges. Crop was sown on May 24, 2013 and May 26, 2014 and thinning was done at third true leaf stage. Fertilizer was applied at 200:115:95 kg N:P:K ha⁻¹ using urea, diammonium phosphate and sulphate of potash as sources. Amount of nitrogen was divided into three equal splits to be applied at time of sowing, 35 days after sowing and 65 days after sowing. Whereas, whole of phosphorus and potash were applied at the time of sowing. All other agronomic practices were kept normal and uniform except treatments under study. Overall 6 irrigations were applied and weeds were controlled by one pre-emergence herbicide at sowing and one post emergence herbicide (with the help of protective shield)

at 50 days after planting. Insecticides were applied as and when required. Crop was harvested each year at its physiological maturity.

Treatments

Heat stress

H₀= No heat imposition

H₁= Heat imposition at square initiation

H₂= Heat imposition at flower initiation

Ascorbic acid levels (Foliar spray)

A₀= Control (water spray)

A₁= 20 mg L⁻¹

A₂= 40 mg L⁻¹

A₃= 60 mg L⁻¹

For imposition of heat stress the plots were covered with polythene sheet for 5 days after the initiation of squares and flowers followed by foliar application of ascorbic acid. Following table shows a difference between temperatures of inside and outside of polythene cover at two reproductive stages of cotton.

Table 1. Variable temperatures during five days of heat imposition at two stages of cotton.

Heat imposition stages	2013		2014	
	Outside polythene cover (natural)	Inside polythene cover (imposed)	Outside polythene cover (natural)	Inside polythene cover (imposed)
Square initiation	37.5°C - 39.8°C	44°C - 47°C	38.4°C - 40.9°C	45°C - 48°C
Flower initiation	38.6°C - 41.2°C	46°C - 48°C	39.5°C - 42.3°C	46°C - 49°C

Following parameters were recorded.

Total number of bolls per plant: Bolls per plant were calculated from already tagged five plants in each plot and averages were taken.

Number of opened bolls per plant: Opened bolls were counted, at first and second picking, of five guarded plants and averaged.

Boll weight per plant: It was obtained by dividing seed cotton yield per plant with respective number of opened bolls per plant.

Number of sympodial branches per plant: These were counted from five tagged plants in each plot and then averaged.

Seed cotton yield per hectare (kg): Seed cotton yield per plot was converted into seed cotton yield per hectare.

Cell membrane thermostability (CMT): The youngest leaves (20 days after unfolding) of the main stem were selected. Leaf discs (10 mm diameter) were taken out, from each side of midrib, with the help of a steel puncher at about 1100 h. Discs were put in the glass vials having 2 ml deionized water and transferred in laboratory. Discs were washed with deionized water, thereafter, 2 ml

double distilled water was added to each vial and capped cotton plug. One set of tubes was placed at 50°C for one hour in a water bath (D9126 Schwabach FRG made in Germany), while the other set (control) was placed at 25°C for the same time. After this treatment, vials were kept at 10°C for 24 hours and then brought to 25°C. Electrical conductivity (EC) meter (GENWAY-4510 conductivity model sr. # 02370, Barlow world scientific limited made in UK) was used to measure EC of these samples. In order to kill the tissues and release all electrolytes, vials were autoclaved (HIRAYAMA HVA-85 manufacturing corporation made in JAPAN, sr. # 31804030007) for 10 minutes at 10 MPa. Final EC was again measured and used the formula given by Sullivan (1972), to calculate relative cell injury (RCI):

$$RCI (\%) = 1 - \left[\frac{1 - \left(\frac{T_1}{T_2} \right)}{1 - \left(\frac{C_1}{C_2} \right)} \right] \times 100$$

Where

T₁ = initial EC value of heat treated vial

C₁ = initial EC value of control vial

T₂ = final EC value of heat treated vial

C₂ = final EC value of control vial

We calculated CMT by deducting RCI % from 100.

Meteorological data: The Meteorological data for the whole growth period of crop were collected (fig. 1) from the Meteorological Observatory, Department of Agronomy, University of Agriculture Faisalabad, Pakistan. The meteorological station is situated about 500 m away from the experimental site.

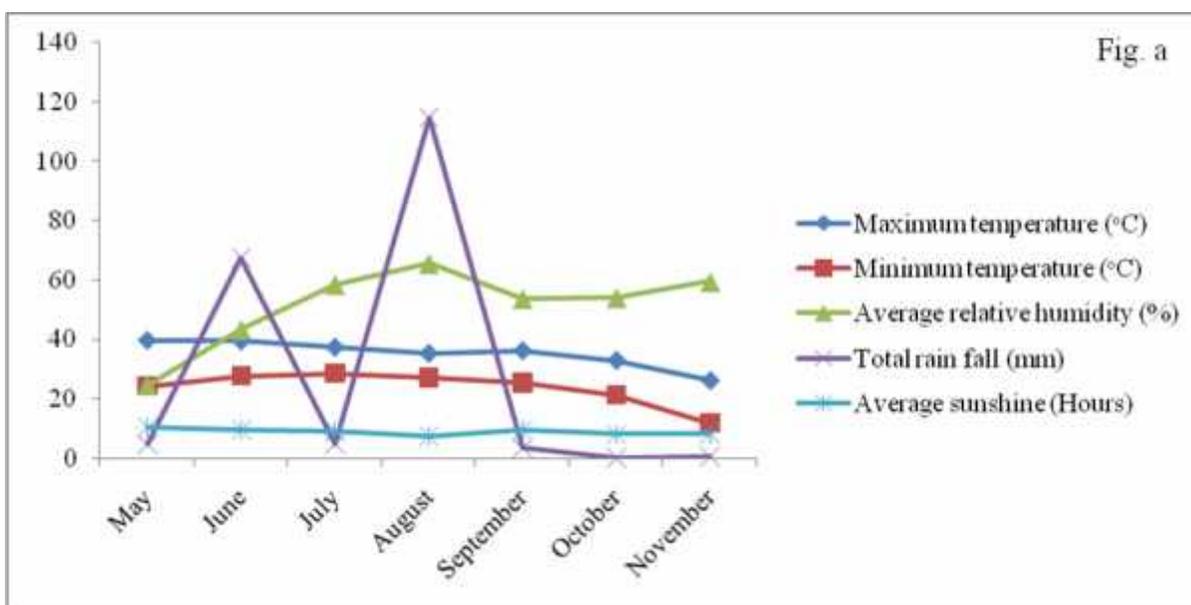
Statistical analysis: The data were analyzed using software STATISTIX 8.1 and the means were compared by Tukey's HSD test at 0.05 probability (Steel *et al.*, 1997).

RESULTS

The treatments' and their interaction ($H \times A$) showed significant effects on total number of bolls per plant, opened bolls per plant and boll weight while the year mean effect was statistically significant on total number of bolls per plant and opened bolls per plant except for boll weight (table 1). More number of bolls (total and opened) was obtained in 2014 than 2013. Heat stress showed significant decrease in total bolls, opened bolls and boll weight as compared to no heat imposition (H_0), either at squaring (H_1) or flowering (H_2), although reduction was more pronounced with respect to flowering stage of cotton crop. Foliar application of ascorbic acid showed beneficial impact under no heat as well as under both heat imposition treatments. By the addition of ascorbic acid there was an increase in the boll number, opened bolls and boll weight and highest values of these parameters were recorded when ascorbic acid was

applied at the rate of 40 mg L⁻¹ (A_2) than control and 20 mg.

Comparison of year means showed higher values for sympodial branches per plant, seed cotton yield per hectare and cell membrane thermostability (CMT) in 2014 than 2013. Treatments' means as well as their interaction ($H \times A$) also showed significant effects on these parameters (table 2). Heat stress, imposed either at square initiation (H_1) or flower initiation (H_2) showed significant reduction in sympodial branches per plant, seed cotton yield per hectare and CMT. Under all the three heat treatments, foliar application of ascorbic acid showed beneficial impact. Foliar applied ascorbic acid improved number of sympodial branches, seed cotton yield per hectare and CMT giving maximum values at 40 mg L⁻¹. However, the plots where heat was imposed at squaring stage (H_1); medium (40 mg L⁻¹) and higher (60 mg ascorbic acid L⁻¹) doses performed equally well for sympodial branches and seed cotton yield per hectare during both years of study. Fig. 2 indicates the association between seed cotton yield per hectare and cell membrane thermostability under regression analysis. The degree of association differed during both years of study. Irrespective of degree of association seed cotton yield showed strong and positive relation with CMT. Mean squares of regression were significant at 1% level of probability. This provided the strong indication that seed cotton yield has solid dependence on membrane stability. The regression points remained scattered close to the regression line during both years of study. The coefficient of association (R^2) was 90 and 84% during first and second year of study, respectively.



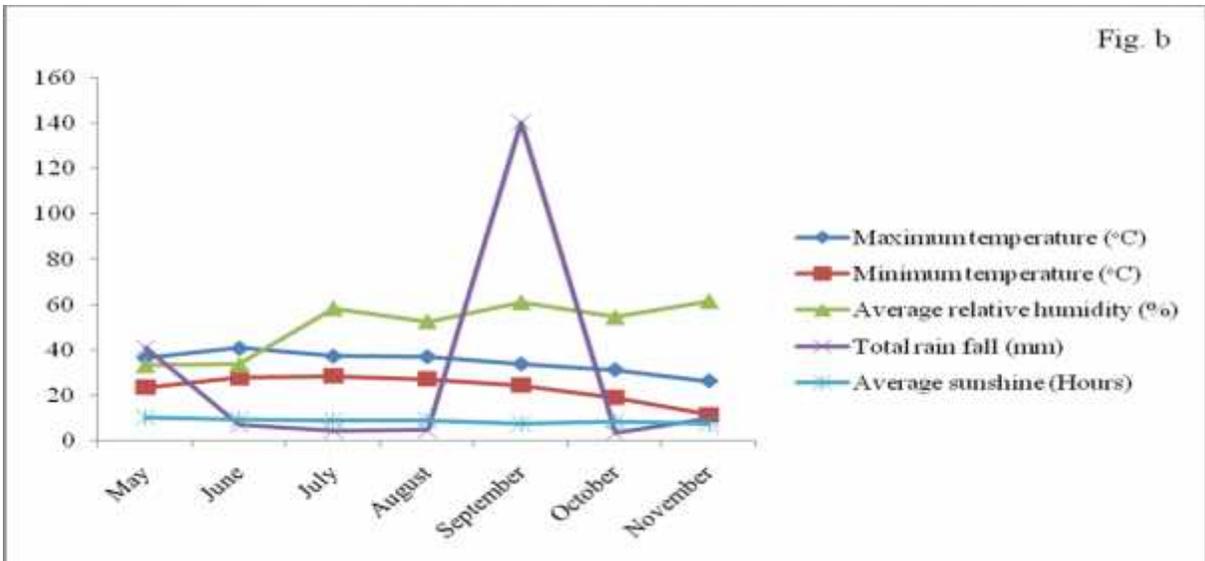


Fig. 1: Weather conditions 2013 (a) and 2014 (b) during cotton crop growth period

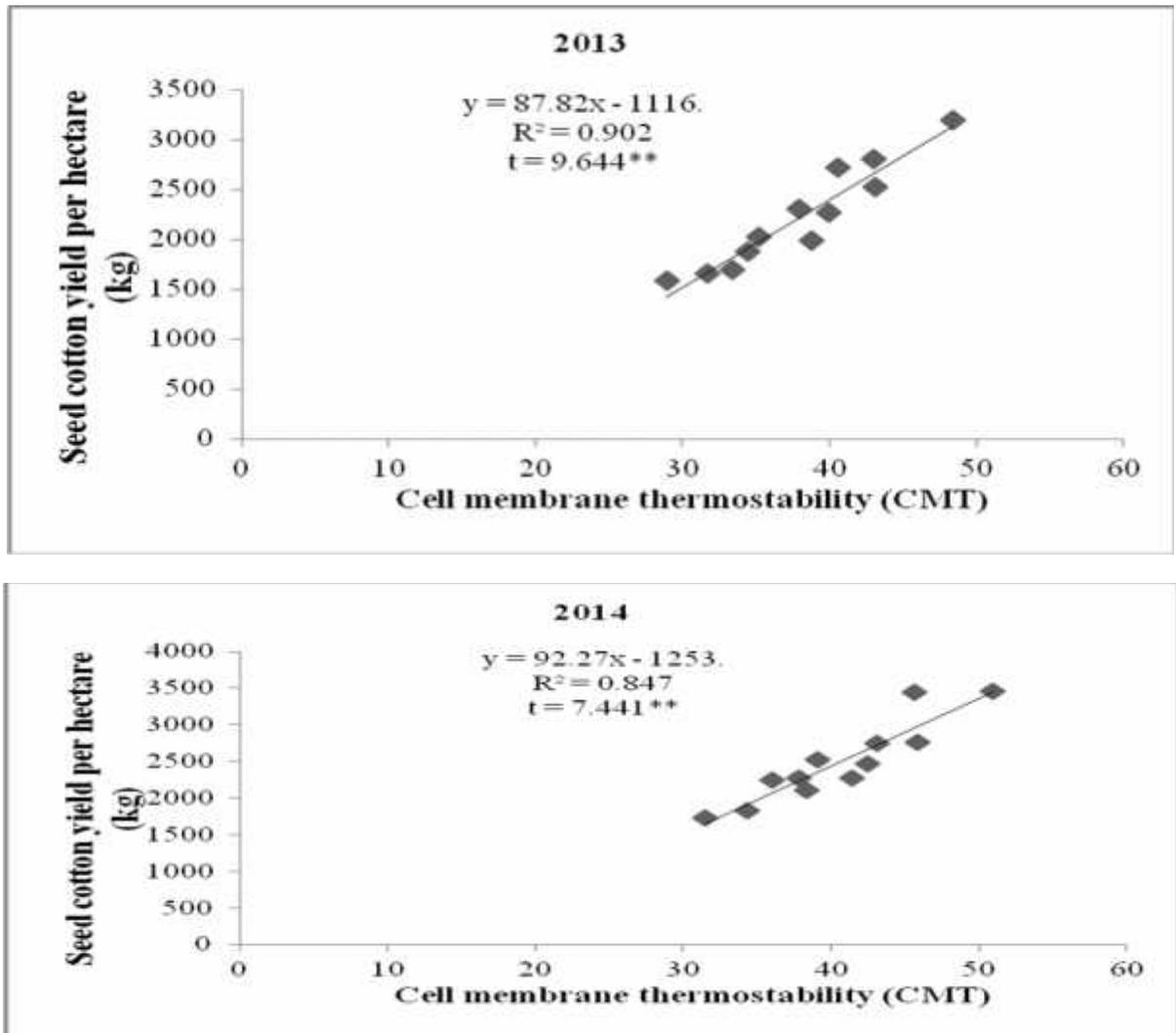


Fig. 2: Relationship between seed cotton yield per hectare (kg) and cell membrane thermostability (CMT)

Table 1. Effect of foliar applied ascorbic acid on total number of bolls per plant, opened bolls per plant and boll weight of heat-stressed cotton

Treatments	Total bolls per plant		Opened bolls per plant		Boll weight (g)	
	2013	2014	2013	2014	2013	2014
No heat imposed (H₀)						
Water spray (A ₀)	31.00 b	34.43 b	23.00 b	26.63 b	2.59 c	2.58 b
20 mg ascorbic acid L ⁻¹ (A ₁)	31.43 b	34.77 b	23.57 b	27.10 b	2.75 b	2.77 b
40 mg ascorbic acid L ⁻¹ (A ₂)	36.37 a	40.87 a	28.77 a	34.00 a	3.14 a	3.03 a
60 mg ascorbic acid L ⁻¹ (A ₃)	35.27 a	36.50 b	28.33 a	28.87 b	2.83 b	3.10 a
Heat imposed at squaring stage (H₁)						
Water spray (A ₀)	26.27 b	27.27 b	19.67 b	21.00 b	2.52 c	2.55 b
20 mg ascorbic acid L ⁻¹ (A ₁)	26.60 b	30.50 ab	20.60 b	24.10 ab	2.57 bc	2.65 b
40 mg ascorbic acid L ⁻¹ (A ₂)	30.27 a	32.07 a	24.67 a	26.80 a	2.91 a	2.90 a
60 mg ascorbic acid L ⁻¹ (A ₃)	27.17 b	31.93 a	21.17 b	25.87 a	2.72 b	2.67 b
Heat imposed at flowering stage (H₂)						
Water spray (A ₀)	23.77 b	26.07 b	16.70 b	19.00 b	2.45 b	2.45 b
20 mg ascorbic acid L ⁻¹ (A ₁)	24.03 b	26.70 ab	17.57 ab	19.37 b	2.49 b	2.50 ab
40 mg ascorbic acid L ⁻¹ (A ₂)	26.90 a	29.93 a	20.63 a	23.97 a	2.78 a	2.71 a
60 mg ascorbic acid L ⁻¹ (A ₃)	24.80 ab	27.00 ab	18.40 ab	20.00 b	2.54 b	2.69 a
Tukey's HSD at p 0.05	2.761	3.843	3.406	3.726	0.151	0.221
Year mean	28.66 b	31.50 a	21.92 b	24.73 a	2.69	2.72
Tukey's HSD at p 0.05	2.039		1.954		NS	

Any two means not sharing a letter in common differ significantly at p 0.05

NS= Non-significant

Table 2. Effect of foliar applied ascorbic acid on sympodial branches per plant, seed cotton yield per hectare and cell membrane thermostability (CMT) of heat-stressed cotton.

Treatments	Sympodial branches per plant		Seed cotton yield per hectare (kg)		CMT	
	2013	2014	2013	2014	2013	2014
No heat imposed (H₀)						
Water spray (A ₀)	19.00 c	20.00 c	2300 c	2527 b	37.97 c	39.13 c
20 mg ascorbic acid L ⁻¹ (A ₁)	22.20 b	23.50 b	2714 b	2750 b	40.57 bc	43.17 b
40 mg ascorbic acid L ⁻¹ (A ₂)	25.67 a	26.97 a	3191 a	3461 a	48.37 a	50.97 a
60 mg ascorbic acid L ⁻¹ (A ₃)	22.43 b	26.87 a	2801 b	3436 a	43.03 b	45.63 b
Heat imposed at squaring stage (H₁)						
Water spray (A ₀)	15.23 b	16.93 c	1875 c	2102 b	34.50 c	38.37 c
20 mg ascorbic acid L ⁻¹ (A ₁)	16.53 b	19.27 bc	1990 bc	2274 b	38.83 b	41.43 bc
40 mg ascorbic acid L ⁻¹ (A ₂)	19.87 a	22.53 a	2522 a	2764 a	43.07 a	45.87 a
60 mg ascorbic acid L ⁻¹ (A ₃)	19.90 a	20.10 ab	2262 ab	2470 ab	39.93 ab	42.43 b
Heat imposed at flowering stage (H₂)						
Water spray (A ₀)	12.33 b	15.10 b	1581 b	1726 b	28.93 c	31.53 c
20 mg ascorbic acid L ⁻¹ (A ₁)	14.07 ab	16.23 b	1657 b	1831 b	31.70 bc	34.30 bc
40 mg ascorbic acid L ⁻¹ (A ₂)	16.20 a	19.93 a	2024 a	2276 a	35.23 a	37.83 a
60 mg ascorbic acid L ⁻¹ (A ₃)	15.13 ab	16.63 b	1692 ab	2244 a	33.43 ab	36.03 ab
Tukey's HSD at p 0.05	2.905	3.160	363.5	425.5	3.503	3.339
Year mean	18.21 b	20.34 a	2217 b	2488 a	37.96 b	40.56 a
Tukey's HSD at p 0.05	1.865		253.9		2.571	

Any two means not sharing a letter in common differ significantly at p 0.05

DISCUSSION

Temperature is one of the most critical environmental factors influencing plant growth and development. Suitability of a crop to a given site is governed not only by threshold temperatures but also the extent of the growing season. Daily or seasonal temperatures should coincide with critical stages of plant development. Although cotton likes heat but temperatures above 36°C have negative effects on its growth, development and yield (Baloch and Lakho, 2000). It is documented that reproductive organs of all plants are usually more sensitive to high temperature stress than vegetative organs (Zinn *et al.*, 2010). Terminal heat (stress during reproductive phase), as in our study, badly affected the process of pollination resulting less number of bolls. It was reported by Snider *et al.* (2009) that high temperatures can reduce boll size and seed cotton yield. Zhao *et al.* (2005) determined that cotton plants exposed to 36/28°C day/night temperature range retained approximately 70% less bolls than those plants which were grown under 30/22°C. According to Naveed *et al.* (2014) both pistillate and staminate flowers in spring sown maize were badly affected by high temperature that ultimately resulted in poor seed setting. The opened bolls per plant under no heat imposition were more only because of more number of total bolls per plant than heat imposition plots. Reduction in sympodial branches might be due to negative effects of high temperature on photosynthesis process of crop, stem elongation etc. Due to low photosynthetic rate, less photosynthates were available for crop growth. Our notion similarly supported the findings of Bibi *et al.* (2010) that, above 35°C, leaf extension rate in upland cotton was reduced significantly. As per findings of Saleem *et al.* (2014) there were more opened bolls and seed cotton yield per plant in earlier planting than late planting because late planted crop faced high temperature during peak flowering. Less seed cotton yield during 2013 than 2014 may be due to less number of total bolls per plant in year 2013 because of rain fall near the boll formation stage (fig. 1) causing fewer bolls to contribute in seed cotton yield. It was proved that heat stress can cause significant pre- and post harvest damages of plant including fruit damage and discoloration and reduced yield (Vollenweider and Goerg, 2005). Photosynthesis is very sensitive to high temperature (Centritto *et al.*, 2011) causing membrane disorder, particularly to membranes of thylakoid, leading to decrease in the activities of membrane-associated enzymes and electron carriers (Rexroth *et al.* 2011) ultimately reducing crop yields. It was clear from our study that under high temperature stress, membrane thermostability (CMT) was reduced to its maximum, because under heat stress reactive oxygen species (ROS) are produced which cause lipid peroxidation resulting in cellular injury leading to reduction in cell membrane

thermostability. It was reported that heat stress can increase the permeability of membranes by altering the structure of membrane proteins and sometimes denature these proteins, as cellular membranes are highly sensitive to stresses (Tayefi *et al.*, 2011). Savchenko *et al.*, (2002) reported that movement of molecules across membranes was accelerated by high temperature stress that led to loosening of chemical bonds due to which lipid bilayer of membranes became more fluid through protein denaturation and increased unsaturated fatty acids. Heat stress enhanced lipid peroxidation of membranes and provoked membrane injury in soybean (Djanaguiraman *et al.*, 2011), cotton (Djanaguiraman *et al.*, 2009) and sorghum (Djanaguiraman *et al.*, 2010).

Ascorbic acid (AA) has beneficial impact on the plants under abiotic stress conditions. Ascorbic acid has an antioxidative role in plant scavenging the reactive oxygen species (ROS) and increasing yield of the crop. Ascorbic acid protected the plants against various environmental stresses like heavy metal, temperature and salinity (Shalata and Neumann, 2001; Vwioko *et al.*, 2008). Ascorbic acid plays multiple roles in many developmental processes including cell division and cell wall expansion leading to improved plant growth (Pignocchi and Foyer, 2003). It was reported that exogenously applied ascorbic acid decreased negative impacts of many stresses such as heat stress in rice (Shah *et al.*, 2011), sunflower (Ebrahimian and Bybordi, 2012), mungbean (Kumar *et al.*, 2011) and wheat (Malik and Ashraf, 2012). Farahat *et al.* (2013) grew *Grevillea robusta* in salt stress condition and found that foliar applied ascorbic acid increased plant height, stem diameter, number of leaves per plant, root length as well as fresh and dry weights of shoots and roots, total carbohydrates, pigments content leading to increase in final yield. Foliar application of ascorbic acid increased leaf area and number of leaves of millet plants grown under salinity stress (Hussein and Alva, 2014). Hussein *et al.* (2011) also proved that ascorbic acid (AA), applied through foliar method, improved the parameters of growth and yield of wheat crop and decreased the salt effect. Beltagi (2008) reported the beneficial effects of ascorbic acid on growth and yield which are due to the antioxidant activity of ascorbic acid protecting plants from abiotic stress. Results of our experiment showed significant increase in CMT by ascorbic acid as ascorbic acid might have increased the tolerance of plants against the oxidative stress. Exogenous ascorbic acid scavenges reactive oxygen species (ROS) increasing the activities of antioxidant enzymes reducing lipid peroxidation. He and Hader (2002) determined that ascorbic acid exhibited a protective influence on the lipid peroxidation under UV-stress. Dolatabadian *et al.* (2009) reported that ascorbic acid, as an antioxidant, has the ability to mitigate the adverse impacts of stress on plants by neutralizing

harmful oxidants which have been reported to damage plant membranes.

Conclusion: Heat stress, at squaring or flowering, caused cell injury that badly impacted the cotton yield and its related components. Foliar applied ascorbic acid at 40 mg L⁻¹ showed considerable improvement in cell membrane thermostability and yield of heat-stressed cotton crop.

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