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COMPARATIVE ABILITY OF SOME GROWTH REGULATORS FOR INDUCING THERMOTOLERANCE IN COTTON UNDER DIFFERENT THERMAL REGIMES

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Heat stress is the most important abiotic factor affecting cotton crop. Exposure of cotton crop to different thermal regimes at different growth stages influences growth and seed cotton yield. The foliar spray of growth regulators at these stages improves yield and growth of cotton crop by mitigating the adverse effects of heat stress. Therefore, a field study was conducted to evaluate the potential role of different growth regulators on thermo-tolerance in cotton during two consecutive years (2012 and 2013). Different thermal regimes were provided at three reproductive stages by overwhelming different temperatures under field conditions (April 2, May 3 and June 17 during 2012 and April 4, May 2 and June 19 during 2013). Foliar spray of water spray (control), hydrogen peroxide (30 ppm), salicylic acid (50 ppm), *Moringa* leaf extract (30 times diluted) and ascorbic acid (70 ppm) were studied at squaring, flowering and boll formation stages. April sown crop produced highest seed cotton yield, plant height, more number of nodes for the first fruiting branch and monopodial branches followed by May and June thermal regimes. June sown crop showed higher unopened bolls than others. Hydrogen peroxide (H_2O_2) produced higher seed cotton yield per plant and monopodial branches in high temperature sowing dates (April and May) than optimal thermal regime (June sown crop). *Moringa* leaf extract (MLE) and ascorbic acid (ASA) also produced similar results under high temperature sowing dates. The H_2O_2 and SA reduced unopened bolls in all thermal regimes over the control while MLE and ASA showed similar results like water spray. All the growth regulators showed non-significant effect on node number for first fruiting branch in all thermal regimes except in April sown crop where H_2O_2 and SA reduced node numbers over the water spray.
Keywords: Growth substances, foliar spray, *Gossypium hirsutum*, high temperature stress, sowing dates, yield.

INTRODUCTION

The climate changes would raise global air temperature by 1.1-6.4°C (Lobell and Field, 2007), and at the end of this century, it would rise up to 1.4 to 5.8°C (Houghton *et al.*, 2001). Hot semi-arid conditions have a negative impact on the production of cotton crop. It is also grown successfully in arid-irrigated regions and semi-humid areas of the world (Hearn, 1994). Cotton crop likes hot climate but very high temperatures affect its growth and yield badly as there has been found a negative relationship between high temperature and the yield of cotton crop (Oosterhuis, 1999). The most favorable temperature for cotton growth and photosynthesis ranges from 20-30°C (Burke *et al.*, 1988; Reddy *et al.*, 1991) while above 35°C cotton growth starts affecting.
Unpredictable year to year diversity in cotton yield is due to water and temperature stresses (Brown *et al.*, 2003; Saleem *et al.*, 2015). High day and night temperature is much responsible for year to year variation in cotton yield (Oosterhuis, 2002). When plant temperature exceeds the optimum, it causes reduction in vegetative and reproductive growth (Singh *et al.*, 2007; Mahmood *et al.*, 2014) leading to reduction in yield of cotton crop (Easterling *et al.*, 2007; Ainsworth *et al.*, 2008).
Very high and low temperatures influence the efficacy of Bt cotton during different growth stages of crop (Mahon *et al.*, 2002). Brown *et al.* (1995) reported that during the monsoon nights, cotton foliage is 4-5°C warmer than the foliage in summer nights. Under field conditions, sowing date is the most important factor for initiation of heat stress. Reproductive stages of early sown cotton come during the hottest months that can cause serious reduction in yield (Rahman *et al.*, 2007; Khan *et al.*, 2014).
Growth regulators are organic or inorganic substances activating plant defensive system under abiotic stresses (Wahid and Shabbir, 2005). Hydrogen peroxide (H_2O_2) and salicylic acid (SA) are useful substances having role towards the release of stress (Gechev *et al.*, 2006). The SA is a growth regulator within the plant body that regulates a number of phsyiological processes (Hayat *et al.*, 2007) that palys an important role in stress tolerance in plants. *Moringa* leaf extract has great importance as its leaves contain

vitamin A and C, iron, calcium, potassium, riboflavin, beta-carotene, zeatin and phenolic acids (Nambiar *et al*., 2005), so it can be used as a growth regulator. Ascorbic acid, present within cell walls, regulates cell division and photosynthesis process and can be used to increase stress tolerance in plants (Ashraf and Foolad, 2007).

All the above mentioned growth regulators are tested in various crops under different stress conditions while limited information is available on use of both organic and in-organic growth regulars against heat stress on cotton. Keeping in view the importance of heat stress, thermal regimes and growth regulators; present study compares different sowing dates for their role in imposing heat stress and investigates the supplement role of growth regulators for alleviating the adverse effects of heat stress on growth, yield and earliness of cotton crop.

MATERIALS AND METHODS

***Location*:** The experiment was conducted during 2012 and 2013 in the field at Students' Farm, Department of Agronomy, University of Agriculture Faisalabad, Pakistan. The soil of the experimental area was sandy clay loam. In field, different sowing times, referred as thermal regimes, provided different temperatures to crop (Pettigrew, 2002).

***Thermal regimes and conditions for the experiment*:** Three sowing times (April 2, May 3 and June 17 during 2012 and April 4, May 2 and June 19 during 2013) providing three temperatures/thermal regimes (optimum, sub and supra-optimal) at squaring, flowering and boll formation stages (through the revision of previous five years' climate data) were selected (Fig. 1**)**. The June sown crop was control as it provided optimal temperature at all reproductive stages while April and May thermal regimes provided sub and supra-optimal temperatures at three reproductive stages of cotton crop. The meteorological data were collected during both years of study from meteorological observatory of Department of Agronomy, University of Agriculture Faisalabad, Pakistan (Fig. 1). Treatments were laid out in randomized complete block design (RCBD) with split plot arrangement keeping sowing times (April, May, June) in main plots and foliar feeding of growth regulators i.e., hydrogen peroxide (H_2O_2 30 ppm), salicylic acid (SA 50 ppm), *Moringa* leaf extract (MLE 30 times diluted) and ascorbic acid (ASA 70 ppm) in sub plots. The doses for each regulator were optimized in the preliminary experiments (data not shown). The foliar spray of growth regulators was applied at squaring, flowering and boll formation stages of three reproductive stages. Experiment was replicated thrice using net plot size of 6.0 m × 4.5 m. Crop was planted on 75 cm apart ridges by manual dibbling and plant to plant distance was maintained at 30 cm. Nine irrigations were applied as per crop requirement keeping in view the reproductive stages under study to avoid drought during heat stress periods. In this experiment, a single medium heat tolerant variety (AA-802) was selected from a preliminary experiment (data not shown) because a heat tolerant cultivar might not show clear response to regulators and the susceptible cultivar might be too much affected to show the effects of regulators.

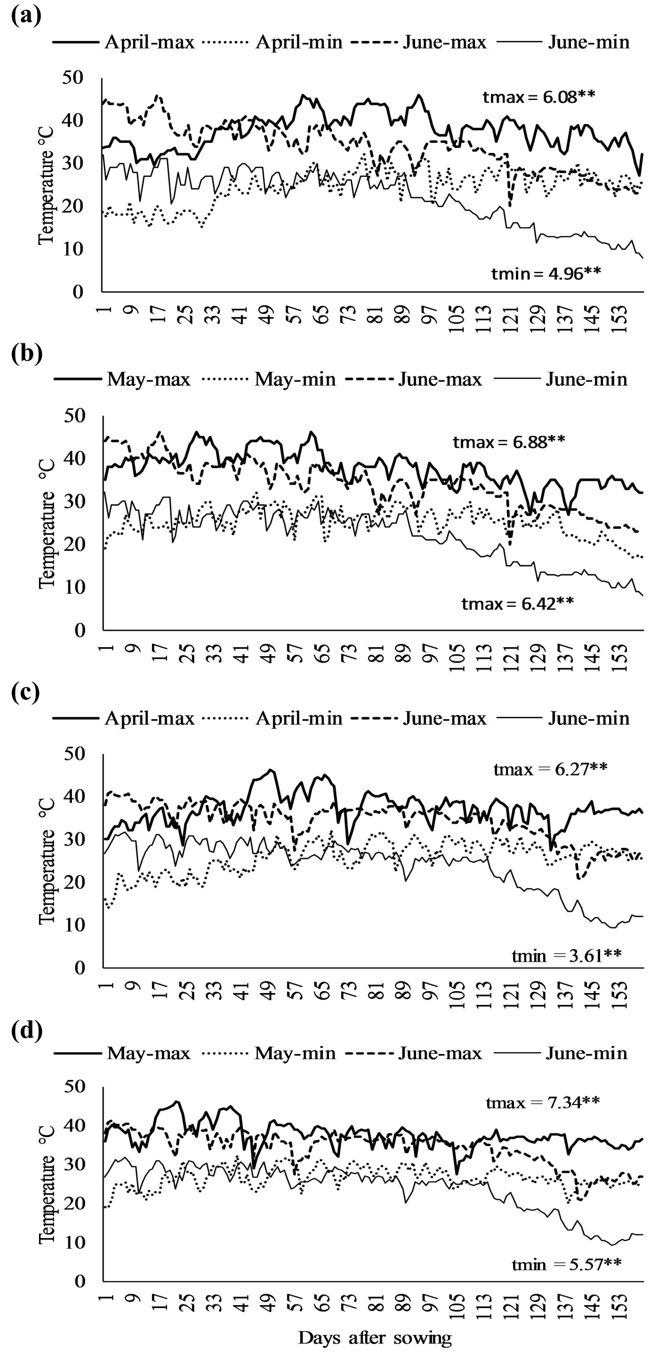


Figure 1. Daily maximum and minimum temperatures for (a) April-June, (b) May-June during 2012 and (c) April-June (d) May-June during 2013.

***Moringa leaf extract (MLE)*:** The young leaves and branches of *Moringa* (*Moringa oleifera*) were taken from fully grown young trees located at the field area of Agronomy Department, University of Agriculture Faisalabad. *Moringa* leaf extraction was done according to the methodology described by Price (2000). Young *Moringa* leaves with tender branches were ground with a small amount of water (1L/10 kg plant material), in a locally designed machine. The extracted material was sieved with cheese cloth, and then centrifuged for 10 min at 8000×g. Ten plants were selected and tagged in each experimental unit for recording data according to standard procedures.

***Statistical procedure*:** Statistix 10.1 program was used for analysis of variance (ANOVA). Before running combined ANOVA, separate ANOVA was run for each sowing date and growth regulator. Coefficient of determination was run separately for yield related and earliness related components. Significance of results was reported at 5 and 1% of probability. Graphs were made by using Microsoft Excel Program.

RESULTS

Different sowing dates affected seed cotton yield per plant, plant height and number of monopodial branches per plant significantly. April sown crop produced significantly higher seed cotton yield per plant (SCY), plant height (PH) and number of monopodial branches per plant during both years of study (Fig. 2-4). May sown crop preceded April sowing while June sown crop represented lowest seed cotton yield,

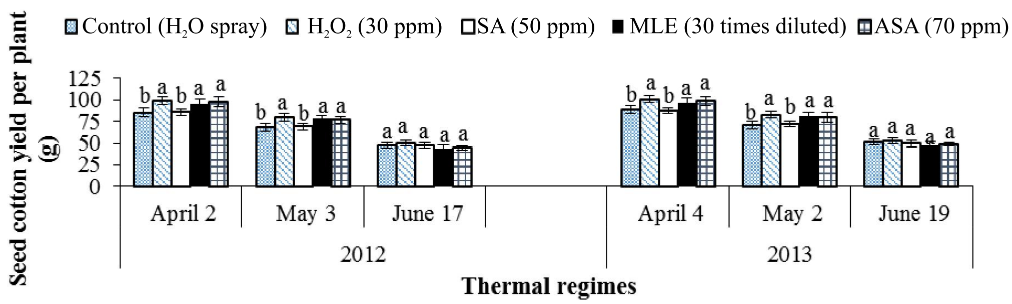


Figure 2. Effect of different thermal regimes and growth regulators' spray on seed cotton yield per plant. Bars are the means ± SE (n=3). Lettering has been done separately on each sowing date using LSD of the interaction table.

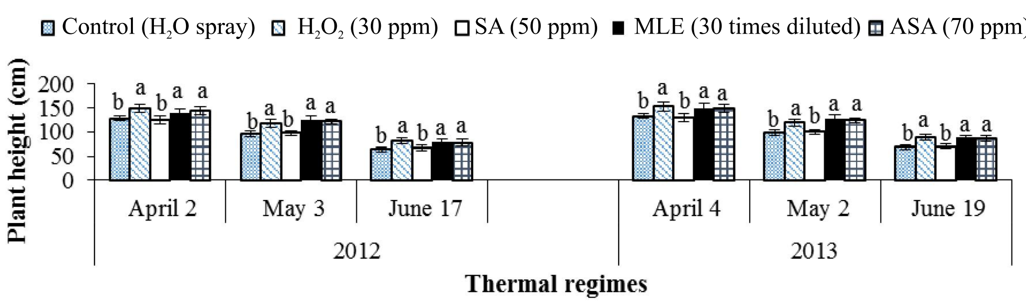


Figure 3. Effect of different thermal regimes and growth regulators' spray on plant height of cotton. Bars are the means ± SE (n=3). Lettering has been done separately on each sowing date using LSD of the interaction table.

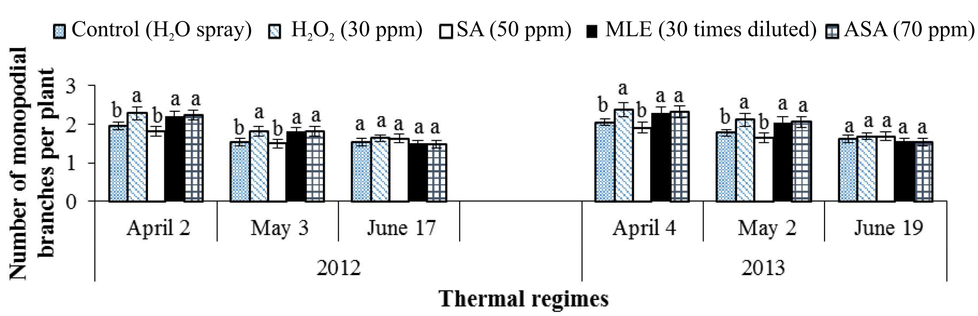


Figure 4. Effect of different thermal regimes and growth regulators' spray on monopodial branches of cotton. Bars are the means ± SE (n=3). Lettering has been done separately on each sowing date using LSD of the interaction table.

plant height and number of monopodial branches during 2012 and 2013. June sown crop during both years of study showed significantly higher unopened bolls, while April sowing indicated the lowest unopened bolls (Fig. 5). Similarly, April sown crop induced higher node numbers for first fruiting branch (NFFB) during both study years while both May and June thermal regimes produced similar node numbers but preceded April thermal regime (Fig. 6). Among different growth regulators, foliar spray of hydrogen peroxide (H_2O_2) produced higher seed cotton yield per plant and number of monopodial branches per plant under April and May thermal regimes over control and SA during both study years while both *Moringa* leaf extract (MLE) and ascorbic acid (ASA) produced statistically similar results with hydrogen peroxide (Fig. 2, 4). Hydrogen peroxide increased seed cotton yield per plant by 15% and monopodial branches by 16.5% in April thermal regime (averaged across both years) over control. Averaged across both years, seed cotton yield per plant was reduced by 25%, number of monopodial branches per plant by 21% in May thermal regime control (water spray) than April regime control. Foliar spray of hydrogen peroxide increased seed cotton yield up to 16.6% and monopodial branches up to 18.5% in May sown crop over the control. It is also clear that all growth regulators brought statistically similar results in optimum temperature sowing date (June). Hydrogen peroxide increased plant height under all thermal regimes than salicylic acid and water spray while both MLE and ASA also produced higher results than control and SA (Fig. 3). High temperature stress reduced plant height in May

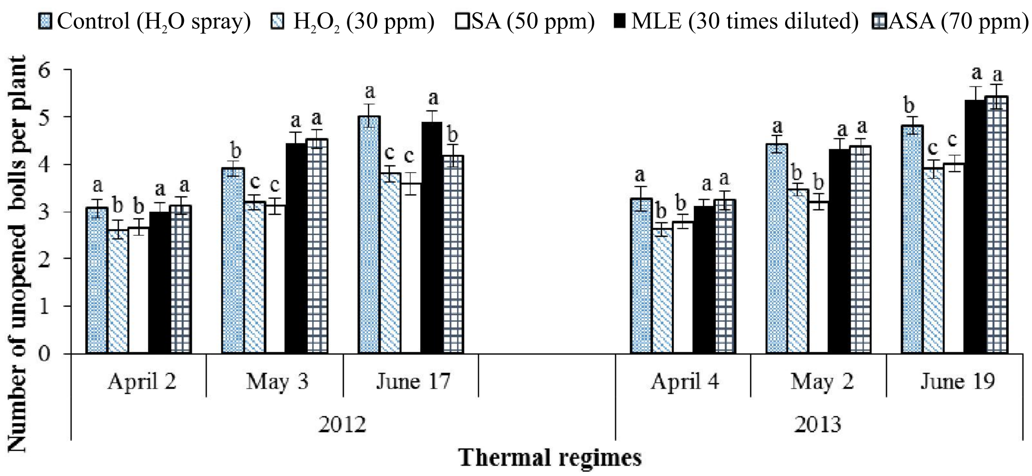


Figure 5. Effect of different thermal regimes and growth regulators' spray on unopened bolls of cotton. Bars are the means ±SE (n=3). Lettering has been done separately on each sowing date using LSD of the interaction table.

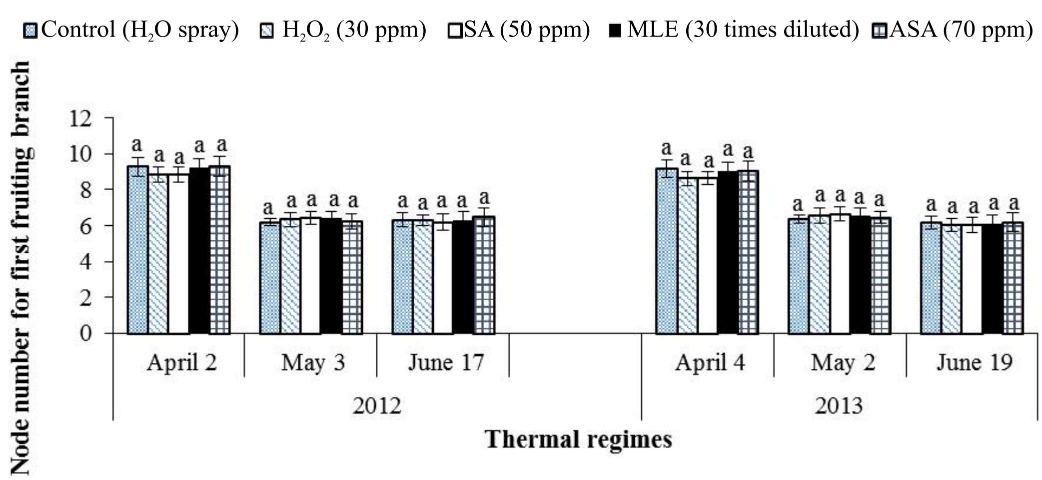
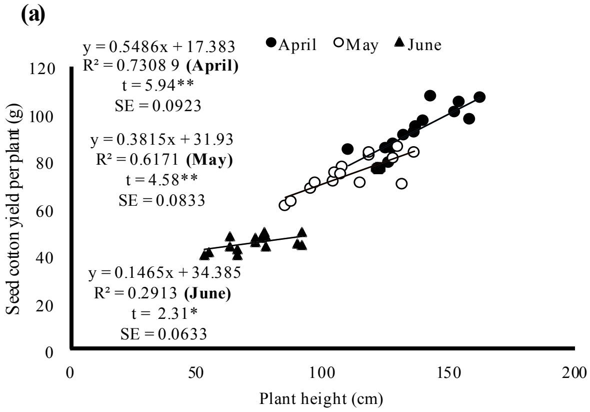


Figure 6. Effect of different thermal regimes and growth regulators' spray on node number for first fruiting branch of cotton. Bars are the means ± SE (n=3). Lettering has been done separately on each sowing date using LSD of the interaction table.

sown control (water spray) up to 34.5% than April regime control while H_2O_2 increased this plant height up to 16% in April sown crop, 22.7% in May sown crop and 28% in June sown crop. Hydrogen peroxide and salicylic acid reduced unopened bolls in all thermal regimes but induced better results in June and May sown crops over the control while MLE and ASA mostly showed higher unopened bolls as control (Fig. 5). All the regulators showed non-significant effects on node number for first fruiting branch during both years of study under all thermal regimes except that H_2O_2 and SA reduced node numbers only in April thermal regime (Fig. 6).

Relationship of plant height (PH), node number for first fruiting branch (NFFB), number of monopodial branches and unopened bolls per plant with seed cotton yield (SCY): The relationships between PH and SCY; NFFB and SCY, monopodial branches and SCY; unopened bolls and SCY were studied under regression analysis (Fig. 7-10).

(a)



(b)

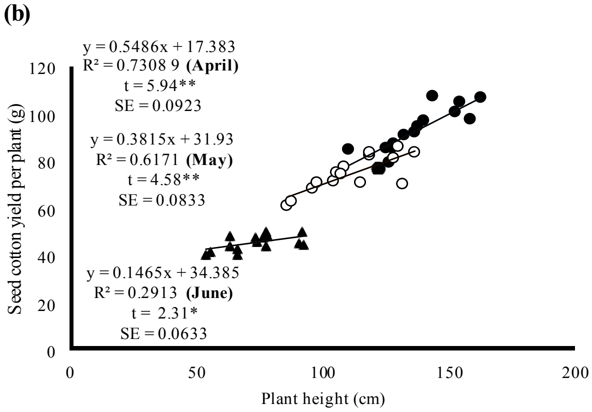
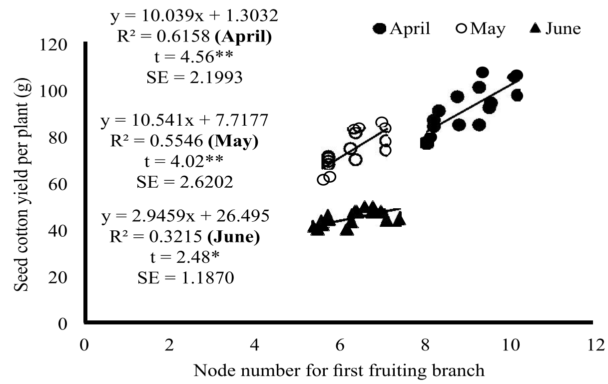


Figure 7. Association between plant height and seed cotton yield per plant under April, May and June sowing dates during 2012 (a) and 2013 (b). * and ** indicate significance at 5 and 1% levels, respectively.

The magnitude of correlation varied for April, May and June thermal regimes. Association of PH, NFFB and monopodial branches with SCY was positive under all sowing dates irrespective of magnitude (Fig. 7-9) while unopened bolls indicated negative association with SCY under all thermal regimes (Fig. 10).

(a)



(b)

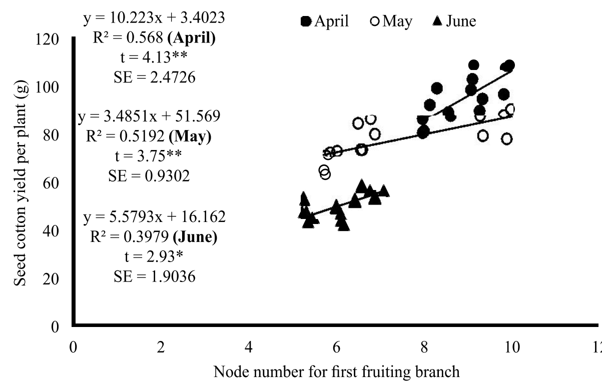


Figure 8. Association between node number for first fruiting branch and seed cotton yield per plant under April, May and June during 2012 (a) and 2013 (b). * and ** indicate significance at 5 and 1% levels, respectively.

Significantly higher association was observed under April thermal regime for PH, NFFB and monopodial branches with SCY followed by May regime while June thermal regime showed lowest association during both study years. Mean squares of regression were significant at $P<0.01$ in April and May regimes while $P<0.05$ in June thermal regime during 2012 and 2013. This provides strong evidence that there is a strong association of PH, NFFB and monopodial branches with SCY under high temperature sowing dates than optimum thermal regimes. Regression analysis indicated significantly higher negative association between unopened bolls and SCY under June sown crop than both May and April thermal regimes. Mean squares of regression were significant at $P<0.05$ only in June thermal regime during 2012 and 2013. Averaged across both study years, the coefficient of determination (R^2) accounted 75% in April, 63% in May and 27% in June sowing date for PH and SCY.

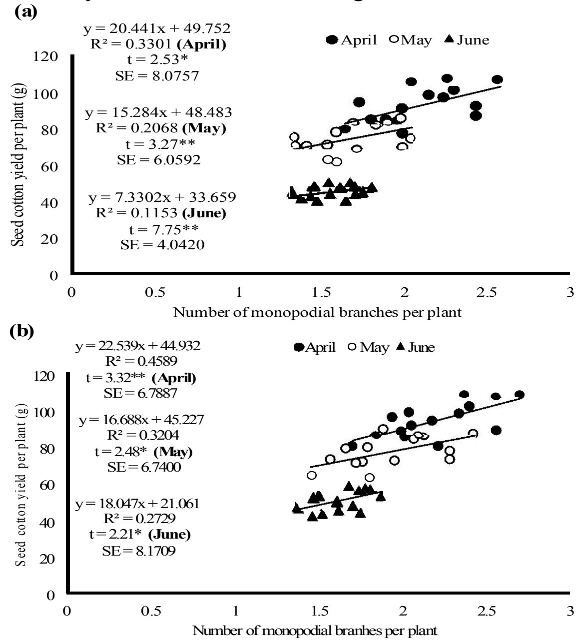


Figure 9. Association between number of monopodial branches per plant and seed cotton yield per plant under April, May and July dates during 2012 (a) and 2013 (b). * and ** indicate significance at 5 and 1% levels, respectively.

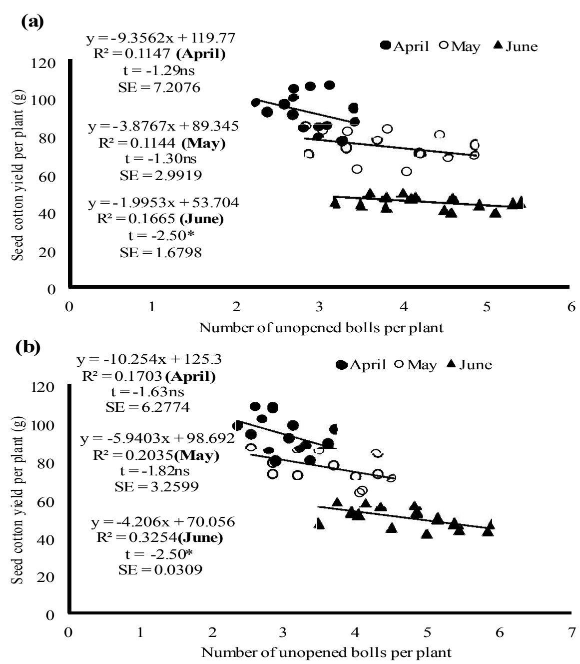


Figure 10. Association between number of unopened bolls and seed cotton yield per plant under April, May and June sowing dates during 2012 (a) and 2013 (b). * and ** indicate significance at 5 and 1% levels, respectively.

***Relationship between NFFB and PH; PH and number of monopodial branches per plant*:** The relationships between NFFB and PH; PH and monopodial branches were studied under regression analysis (Fig. 11, 12). The degree of correlation varied for April, May and June thermal regimes. Associations between NFFB and PH; PH and monopodial branches were positive under all sowing dates irrespective of degree of association. Regression analysis showed significantly higher association under April and May thermal regimes over the June thermal regime. Mean squares of regression were highly significant (P<0.01) in April and May regimes while significant (P<0.05) in June thermal regime during both study years. This provides strong evidence that there is strong association between NFFB and PH; PH and monopodial branches under high temperature sowing dates than optimum thermal regime.

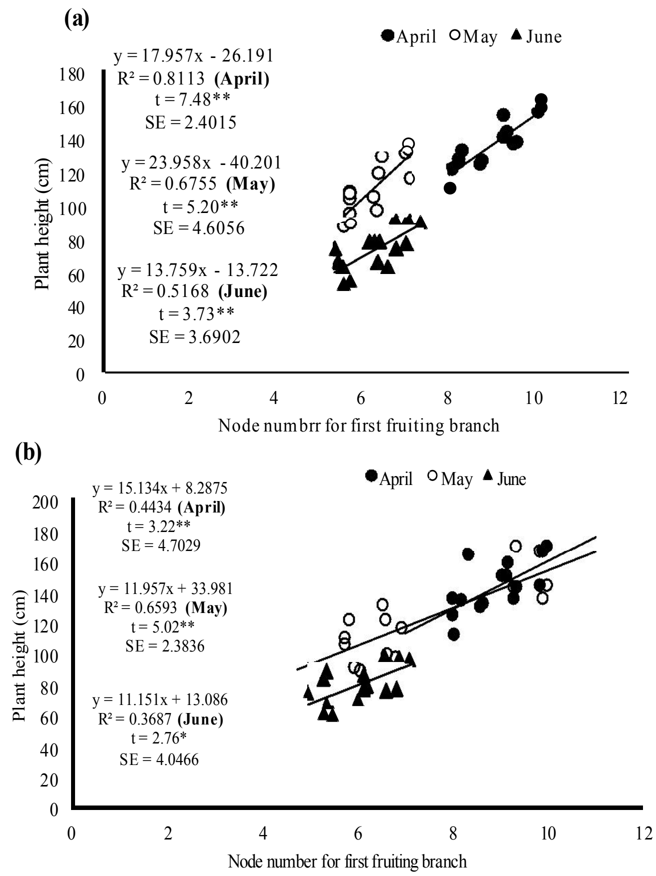


Figure 11. Association between node number for first fruiting branch and plant height under April, May and June sowing dates during 2012 (a) and 2013 (b). * and ** indicate significance at 5 and 1% levels, respectively.

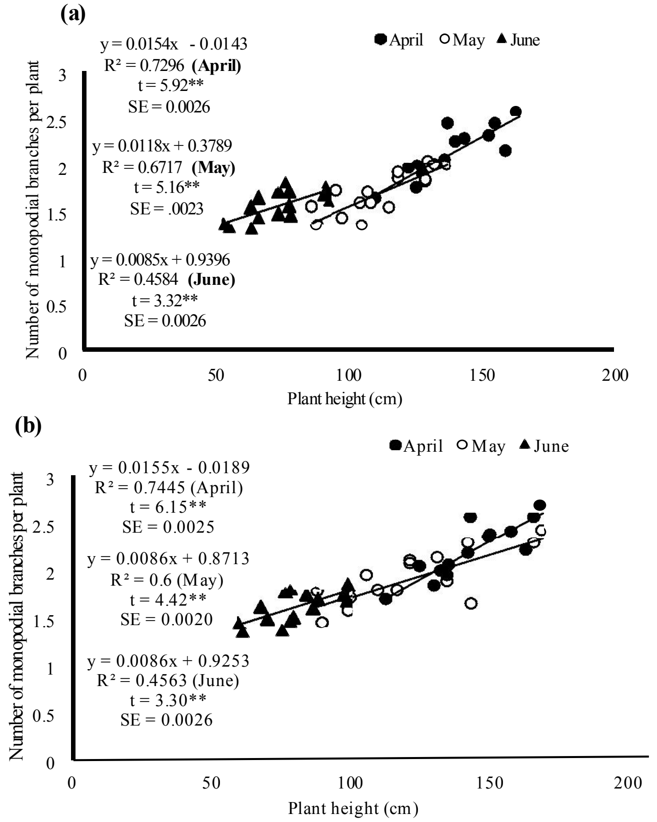


Figure 12. Association between plant height and number of monopodial branches per plant under April, May and June sowing dates during 2012 (a) and 2013 (b). * and ** indicate significance at 5 and 1% levels, respectively.

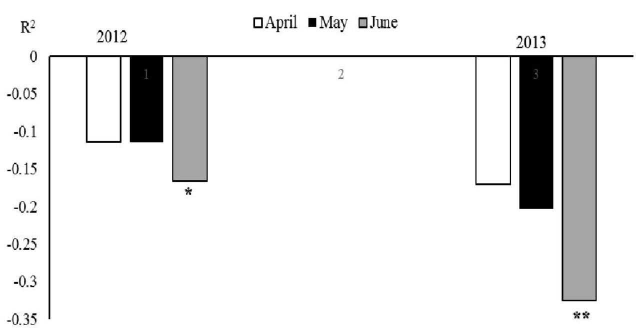


Figure 13. Association between unopened bolls and seed cotton yield per plant under April, May and June sowing dates during 2012 and 2013.

DISCUSSION

Pettigrew (2008) observed that when temperature exceeds 1°C from ambient air temperature (35 to 38°C) during three years of study, the warm regime reduced lint yield by 10% supporting the work of Burke *et al*. (1988) that 32°C is the optimum temperature for cotton crop. Conaty *et al*. (2012) found cotton yield reduction when temperature was increased above 30°C. Cottee *et al*. (2008) examined yield reduction under heat stress regime (artificially created) over the ambient temperature of field conditions where temperature remained above 35°C throughout cotton growing season. Early plantation of cotton increased 10% yield of cotton crop, due to early flowering and early canopy development intercepting more sunlight and attained more plant height (Pettigrew, 2002). In the present study, the seed cotton yield was decreased more in the controls of high temperature sowing dates than other regulators over the June thermal regime. But the April and May sown crops produced higher yield than June thermal regime. April sown crop at early growth stages faced favorable temperature (Fig. 1) leading towards heat acclimation. Although June thermal regime experienced a favorable temperature at different reproductive stages but it availed less growing degree days (GDD) at its later stages so, could not attain the required GDD for boll opening (than April and May sown crops) leading towards poor boll opening and thus less yield (April sown crop took 2449 and 2446 GDD, May took 2537 and 2447 GDD and June took 2257, 1961 GDD during 2012 and 2013). The June sown crop also faced higher temperature and accumulated higher heat units at its seedling stages than April and May sown crops. As the late sown crop faced less heat stress during different reproductive stages therefore, the effects of foliar applied regulators were non-significant on this crop. Hydrogen peroxide (having role in growth and cell signaling) increased seed cotton yield per plant, plant height and number of monopodial branches in high temperature sowing dates (April and May) than optimum (June) sowing date but the effect was more pronounced in May sown crop having more heat stress at its growth stages. Similarly, MLE (having role in growth due to zeatine) and ASA (having role in cell division and growth) increased yield and related components under April and May sown crops over the control and June sown crop. This indicates that growth regulators work well under high temperature conditions by mitigating its adverse effects.

Early plantation of cotton resulted into higher plant growth and seed cotton yield (Pettigrew, 2002; Rauf *et al*., 2004) than late sowing (Bange and Milory, 2004; Davidonis *et al.,* 2004). In this study, June sowing date brought more unopened bolls due to low temperature at boll opening stage over May and April plantation (Fig. 5). Plant growth hormones are the organic substances synthesized in one part and translated to the other plant parts. A very small amount of hormone is required in signaling transduction pathways. These hormones activate an enzyme producing many secondary substances which work as secondary messengers in signaling process. These secondary substances may activate or inactivate other enzymes. Hydrogen peroxide has its role towards the release of stress so, it plays role in plant growth and development (Gechev *et al*., 2006). Hydrogen peroxide works through signaling process so, might be helpful under high temperature conditions (Mittler, 2004). Salicylic acid is a well-recognized growth hormone within plant system (Cothren and Oosterhuis, 2010). Plants treated with MLE as foliar spray increased yield of fruit plants by 20-35% (Makkar and Becker, 1996). Foliar spray of salicylic acid had non-significant effects on seed cotton yield, total number of bolls and on boll size (Heitholt *et al*., 2001) against the early workers (Singh and Kaur, 1980). While foliar spray of ascorbic acid increased grain yield and yield components in wheat crop under salinity stress (Farouk, 2011). In the present study, hydrogen peroxide increased growth and seed cotton yield under heat stress conditions due to its role in growth and in signaling process. Both H_2O_2 and SA produced lesser unopened bolls, indicating their role in earliness over MLE and ASA. Hydrogen peroxide through signaling process while MLE through zeatine and ASA through cell division are involved in plant growth and development process, leading to increased plant height.

Likewise, April regime took more NFFB due to early lower accumulation of heat units leading crop to heat acclimation while May and June sown crops took more heat units earlier. In this study, both SA and H_2O_2 increased crop earliness (reduced NFFB) in April sown crop due to their strong signaling process under abiotic stresses leading to ineffectiveness of growth regulators under May and June sown crops.

***Conclusion*:** Early sowing (April) produced higher seed cotton yield, plant height and monopodial branches while lower unopened bolls than May and June sowing dates. Similarly, April sown crop induced higher nodes for first fruiting branch. Foliar spray of H_2O_2 (30 ppm) at three reproductive stages increased seed cotton yield, plant height and monopodial branches only in high temperature sowing dates (April and May) followed by foliar spray of ASA (70 ppm) and MLE (30 times diluted). Hydrogen peroxide and salicylic acid (50 ppm) reduced unopened bolls in all sowing dates while H_2O_2 and SA reduced NFFB only in April sown crop.

Acknowledgements

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