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Influence of 4-CPA and GA_3 on physiological, biochemical and yield attributes of tomato under high-temperature conditions

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Abstract

The present research investigated the impact of plant growth regulators in mitigating the effects of heat stress in tomato (*Solanum lycopersicum* L.) genotype LST-6 and cultivar Punjab Varkha Bahar-4. In north India, the temperature in the summer season ranges between 25-45 ºC and temperature above 28 ºC leads to heat stress in plants and negatively affects the reproductive stage of plants. Considering this, we subjected the plants to varying concentrations of GA_3 (10, 20, 30 µg/mL) and 4-CPA (15, 45, 75 µg/mL). GA_3 application took place three weeks after transplanting, while 4-CPA was administered during the anthesis stage. We recorded observations from both control and treated plants, with a 10-day gap between each spray treatment. The application of plant growth regulators (PGRs) enhanced the plants' ability to withstand high temperatures by improving photosynthetic efficiency, as evidenced by increased chlorophyll and carotenoid levels in the leaves. The level of different biochemical constituents (total protein, starch, total soluble sugars, phenol and proline content) also increased in PGRs treated plants. Application of GA_3 and 4-CPA also enhanced the membrane thermostability and reduced lipid peroxidation. The PGRs treated plants exhibited increased plant height, leaf area, pollen viability, fruit set, number of fruits per plant and fruit weight, ultimately improving yield. GA_3 and 4-CPA application also increased the total soluble solids, lycopene content and titratable acidity in tomato fruits. Thus, overall improvement was observed with the application of PGRs; however, 75µg /mL 4-CPA was most effective in imparting thermo tolerance.

Key words: *Solanum lycopersicum*, heat stress, GA_3, 4-chlorophenoxyacetic acid (4-CPA), thermotolerance, yield

Introduction

Tomato, member of family Solanaceae, is an important crop in India because its production ranks second globally. About 11% of the world's total tomato crop comes from India (Pavani *et al.,* 2020). It is grown as a vegetable crop and a cash crop. Temperatures beyond the ideal threshold cause heat stress in tomato plants, which prefer temperatures between 20 and 28 °C for growth. Heat stress is caused by the summer season temperature range of 25-45 °C in north India. One of the main abiotic factors that affect tomato plants' output is heat stress, which has a negative impact on physiological and biochemical processes (Abdalla *et al.,* 2020). Heat stress slows down plant development, leaf area, biomass, production of flower buds, fruit formation *etc.* The reproductive stage of plant is more vulnerable to heat stress than vegetative stage (Carmody *et al.,* 2020). The high-temperature stress mainly reduces the yield by reducing pollen viability, fruit set (%) and fruit size. The main non-reproductive processes negatively impacted by high temperatures include photosynthesis, and enzyme activity, especially those engaged in food metabolism, cell membrane stability, *etc.* (Bita and Gerats, 2013).

The primary impact of heat stress on photosynthesis is the inhibition of crucial enzymatic activity, disruption of lipid chains, and elevation of electrolyte loss. The reduction in plant reserves resulting from the limitation of photosynthesis leads to a decline in the sweetness of fruits and vegetables. High temperature also reduces nutrient uptake, negatively affecting biochemical processes (Giri *et al.,* 2017). Heat stress leads to oxidative stress, which results in the formation of reactive oxygen species (ROS) like O_2^-, OH^- *etc.* ROS denatures the proteins, breaks lipid chain (which increases membrane fluidity) and breaks the strands of DNA, ultimately resulting in cell death. To counteract the effects of high temperatures, plants have developed a variety of defenses, including the production of heat shock proteins and antioxidant enzymes, as well as the closing of stomata to limit water loss (Das and Roychoudhury, 2014). The antioxidant enzymes such as glutathione reductase, peroxidase, and superoxide dismutase assist plants in reducing oxidative stress. Ahmed *et al.* (2021) claimed that some PGRs can be useful for conferring thermotolerance to plants. Auxins at low concentrations reduce fruit abscission by promoting tissue attachment by enhancing differentiation and development of vascular bundles. Auxins also increase flowering, cell division *etc.* (Pramanik *et al.,* 2018). Also, GA_3 application promotes stem elongation, promote flowering, increases fruit set %, enhances fruit weight, *etc.* (Ahmed *et al.,* 2022). However, there is a dearth of thermo-tolerant varieties in tomato. It becomes very difficult for farmers to fulfil market demand for tomato during the summer when the temperature reaches 40-45°C, especially in North Indian plains. Considering the information mentioned above, employing plant growth regulators (PGRs) to safeguard tomato plants against the adverse effects of heat stress is plausible. Therefore, the current study was conducted to examine the effects of 4-CPA and GA_3 treatments on the tolerance of tomato plants to temperature stress and, consequently, their overall yield.

Materials and methods

Plant material, growth conditions and application of plant growth regulators (4-CPA and GA_3): The crop was exposed to heat stress under natural conditions when the temperature raised upto 36 °C (Table-1). This experiment was conducted on tomato genotype LST-6 and compared with a heat-tolerant cultivar Punjab Varkha Bahar-4 (PVB-4), at Vegetable Research Farm and laboratories of the Department of Botany, Punjab Agricultural University, Ludhiana. For the experiment, sowing was done in first fortnight of January 2021 and transplanting was done during last week of February 2021. All the field management practices were followed according to the Package of Practices for cultivation of Vegetables, Punjab Agricultural University, Ludhiana. The foliar application of GA_3 (10, 20 and 30 µg/mL) was done after 3 weeks of transplanting *i.e.* at 21 DAT and 4-CPA (15, 45 and 75 µg/mL was done at anthesis stage *i.e.* at 24 DAT. There were 3 replications per treatment and the treatments were arranged in randomized block design. Observations were recorded at an interval of 10 days after spray treatment and the data presented in tables depict the mean values. The data was analysed using SAS software.

Table 1. Temperature and relative humidity from January to June (2021)

Month (2021)	Temperature (˚C)		Relative Humidity(%)	
	Maximum	Minimum	Morning	Evening
January	16.9	7.1	94	65
February	23.8	10.2	93	54
March	29.5	14.9	82	37
April	34.2	16.9	59	20
May	36.3	22.6	57	32
June	36.3	25.3	68	42

Determination of morpho-physiological parameters: Morpho-physiological parameters included plant height, leaf area, membrane thermo stability, total chlorophyll, and carotenoid content. Plant height, leaf length and breadth were measured using scale. Membrane thermo stability was measured by dipping the third leaf of plant in 15 mL distilled water and conductivity was measured after 20 h. Then samples were boiled for 20 minutes and then again conductivity was measured after cooling.

Membrane thermo stability = 100 - electrolyte leakage (%), where

$$\text{Electrolyte leakage} = \frac{\text{CAB-CBB}}{\text{CAB}} \times 100$$

CAB=Conductivity after boiling, CBB=Conductivity before boiling

Leaf chlorophyll and carotenoid content were measured by dipping 0.05g of fresh leaf sample in DMSO followed by heating in incubator at 65 °C for 4 h. After cooling, the absorbance was read at 480 nm, 645 nm and 665 nm. The total chlorophyll and carotenoid content was calculated using following equations
Total Chlorophyll content: $[20.2 \times A_{645} + 8.02 \times A_{665}]$

Total Carotenoid content: $(A_{480}) + 0.114 (A_{665}) - 0.638 (A_{645})$

Determination of biochemical parameters: It included total proteins, total phenols, total soluble sugars, starch, proline content and lipid peroxidation in leaves. Procedure given by Lowry *et al.* (1951) was followed to measure total protein content and was expressed in mg/g dw. Phenol content was determined by following the method given by Swain and Hills (1959) and absorbance was recorded at 630 nm. Total soluble sugars content was measured in mg /g by following the method given by Dubois *et al.* (1956). Starch content was estimated according to the procedure given by McCready *et al.* (1985) and was expressed in mg /g dw. Proline content (mg /g FW) was estimated according to Bates *et al.* (1973) and Lipid peroxidation was measured as malondialdehyde (MDA content) by following the method described by Dhindsa and Matowe (1981).

Determination of quality and yield attributes: Number of fruits per plant was calculated by adding all the number of fruits harvested in entire season from a plant. For pollen viability, anthers were crushed to get pollen grains and then a drop of aceto-carmine stain was added to pollen grains and then slide was observed under microscope. Only darkly stained pollens were considered as viable. Total soluble solids were measured by placing few drops of tomato juice on hand reflectometer. Lycopene content was measured by mixing 2 g of tomato puree with a solution containing hexane, acetone and ethanol in ratio 2:1:1 followed by addition of 0.05% (w/v) butylatedhydroxytoluene. After 15 minutes, distilled water was added and the setup was left undisturbed for the separation of polar and non -polar layer. Hexane layer was used to measure absorbance at 530 nm.

Lycopene (mg/kg) = A503 * 171.7/W

Where, W = Exact weight of tomato added, in grams

Titratable acidity was measured by titrating 10g of tomato puree (diluted with 50 mL deionised water) with 0.1N NaOH to pH8.

$$\text{Titratable acidity} = \frac{\text{NaOH (mL) used x 014878}}{\text{weight of aliqout}} \times 100$$

For calculating fruit set %, 10 flowers in each replication of each treatment were tagged and there fruits were harvested and its percentage was calculated accordingly. Average fruit weight of harvested fruits was measured in grams. Total yield per plant was measured by adding the total weight of fruits harvested form single plant and it was expressed as g/plant.

Results

Effect of plant growth regulators on morpho-physiological traits: Application of growth regulators (4-CPA and GA_3) positively affected various morpho-physiological parameters in genotype LST-6 as well as cultivar PVB-4. 75 µg/mL 4-CPA and

Table 2. Effect of plant growth regulators on morpho-physiological parameters in Punjab Varkha Bahar-4

Treatment	Plant height (cm)	Leaf area (cm^2)	Membrane thermostability in leaves (%)	Chlorophyll content(mg/gm FW)	Carotenoid content(mg/gm FW)
Conc. of GA_3					
10 µg/mL	56.75^{a}	3.75^{jih}	82^{bac}	2.06^{def}	0.12^{ab}
20 µg/mL	61.96^{a}	4.80^{gdfce}	85^{bac}	2.13^{de}	0.13^{ab}
30 µg/mL	66.53^{a}	6.88^{b}	91.8^{a}	3.23^{b}	0.15^{ab}
Conc. of 4-CPA					
15 µg/mL	61.66^{a}	4.73^{gdfceh}	84.4^{bac}	2.04^{dgef}	0.13^{ab}
45 µg/mL	62.13^{a}	5.55^{dc}	88.2^{ba}	2.33^{d}	0.14^{ab}
75 µg/mL	68.05^{a}	7.15^{a}	93.8^{a}	3.82^{a}	0.16^{a}
Control	55.08^{a}	3.03^{jlk}	75.8^{bdac}	1.46^{gh}	0.11^{b}

Values represented by same letters are not significantly different as per Tukey's test ($P<0.001$). Values are the mean values of different stages

Table 3. Effect of plant growth regulators on morpho-physiological parameters in LST-6

Treatments	Plant height (cm)	Leaf area (cm^2)	Membrane Thermostability in leaves (%)	Chlorophyll Content(mg/gm FW)	Carotenoid content(mg/gm FW)
Conc.of GA_3 (µg/mL)					
10	49.86^{a}	3.96^{gjfih}	63.0^{dc}	1.67^{gef}	0.11^{b}
20	50.29^{a}	4.97^{dfce}	70.2^{bdac}	1.83^{dgef}	0.12^{ab}
30	62.17^{a}	5.16^{dce}	76.6^{bdac}	1.93^{dgef}	0.14^{ab}
Conc. of 4-CPA (µg/mL)					
15	56.26^{a}	3.83^{gjih}	67.6^{bdc}	1.72^{gef}	0.12^{ab}
45	58.19^{a}	4.04^{gjfieh}	73.4^{bdac}	1.83^{dgef}	0.13^{ab}
75	62.90^{a}	5.51^{dc}	82.6^{bdac}	2.27de	0.15^{ab}
Control	56.29^{a}	2.28^{l}	59.4^{d}	0.93^{h}	0.10^{b}

Values represented by same letters are not significantly different as per Tukey's test ($P<0.001$). Values are the mean values of different stages

30 µg/mL GA_3 application were most effective (Table 2 and 3). The maximum mean plant height and leaf area was observed in 75 µg/mL 4-CPA treated LST-6 plants and it was 7.56 % and 27.97 % more than in PVB-4 plants. The increase in plant height and leaf area may be attributed to the role of auxins and gibberellins in cell division and cell elongation.

According to Bhattarai *et al.* (2022), application of GA_3 promotes cell division and enlargement in sub-apical meristem. Foliar application of gibberellins leads to taller plants as it stimulates the cell elongation. Along with this it also increases cell permeability which enhances the flow of water and soluble substances within the cell, thus it increases the cell size. Similar results of increase in plant height and leaf area with the application of auxins and GA_3 were stated by Ahmed *et al.* (2022) in tomato. Application of PGRs helped in reducing the electrolyte leakage by increasing the stability of cell membranes in leaves. In LST-6, the recorded maximum mean membrane stability was 93.8 % (75 µg/mL 4-CPA) followed by 91.8 % (30 µg/mL GA_3). While in PVB-4 it was 82.6 % (75 µg/mL 4-CPA) and 76.6 % (30 µg/mL GA_3). Increased membrane stability in PGR treated sunflower plants has been reported by Zayed *et al.* (2017). The application of PGRs also increased chlorophyll, carotenoid, and photosynthetic efficiency in leaves. The leaves of LST-6 possessed higher chlorophyll (3.82 mg/g FW) and carotenoid content (0.16 mg/g FW) in leaves (which ultimately also increased the photosynthetic efficiency in leaves) as compared to PVB-4. The role of auxins in increasing photosynthetic efficiency by increasing the activity of enzymes involved in dark reactions is well established (Khatoon *et al.,* 2020). According to Guo *et al.* (2022) application of GA_3 reduced electrolyte leakage and enhanced the synthesis of antioxidants in chloroplasts which helped in the scavenging of ROS and reduced oxidative damage.

Effect of plant growth regulators on biochemical parameters: Foliar application of PGRs resulted in increased biochemical constituents in leaves *viz.,* total soluble sugars, total starch, total soluble proteins, total phenols and proline content. Among the different concentration of GA_3 and 4-CPA, 30µg/mL GA_3 and 75µg/mL 4-CPA was most effective in all aspects. The genotype LST-6 possessed maximum mean total soluble sugars (96.93 mg/g dw), total soluble proteins (15.20 mg/g dw), total phenol (3.14 mg/g dw), and proline content (0.92 mg/g dw) in leaves with the treatment of 75 µg/mL 4-CPA. However, the maximum mean total starch (233.37 mg/g dw) was observed in 75 µg/mL 4-CPA-treated plants of PVB-4. The application of PGRs reduced the lipid peroxidation in leaves, which was measured in terms of MDA content, thereby stabilizing the membrane. The recorded lipid peroxidation in leaves with the treatment of 75 µg/mL 4-CPA in LST-6 was 0.3 nmol/g FW and in PVB-4 was 0.48 nmol/g FW.

The results obtained in our study are concomitant with those obtained by Muthulakshmi and Pandiyarajan (2013), who also reported an increase in total soluble sugars in *Catharanthusroseus,* following the application of PGRs. An increase in protein content in the leaves of mung bean has been reported with the application of auxins and GA_3 (Islam *et al.,* 2021). The increase in total soluble proteins, total soluble sugars and starch content may be attributed to increased metabolic activity with the application of PGRs. According to Sharma *et al.* (2019) phenols play key role in cell division, hormonal regulation, photosynthesis and nutrient assimilation. Under abiotic stress conditions, the concentration of polyphenols increased, which helped the plant cope with stressful conditions and the application of PGRs also enhanced phenol content in marigold (Sardoei and Shahdadneghad, 2014). The increase in proline content with the application of GA_3 and auxins in tomato and garden peas was observed by Guo *et al.* (2022) and Sergiev *et al.* (2018). According to Harsh *et al.* (2016), plants tend to accumulate more proline content which helps to stabilize enzymes, maintain membrane integrity and scavenge ROS under abiotic stress conditions. Application of PGRs increased the membrane stability and antioxidant activity and reduced the lipid peroxidation in *Phoenix dactylifera,* as reported by Khan *et al.* (2020)

Effect of plant growth regulators on yield and quality attributes: Foliar application of plant growth regulators (PGRs) significantly impacted the yield and quality attributes of LST-6 and PVB-4 tomato cultivars, as demonstrated in Tables 4 and 5. The application of 30 µg/mL gibberellic acid (GA_3) and 75 µg/mL 4-chlorophenoxyacetic acid (4-CPA) resulted in comparable outcomes in terms of yield and quality attributes for both cultivars.

The maximum mean number of fruits per plant was observed in LST-6 when treated with 75 µg/mL 4-CPA. However, PVB-4 displayed superior performance in several key attributes when treated with 30 µg/mL GA_3 and 75 µg/mL 4-CPA. Specifically, PVB-4 exhibited the maximum mean pollen viability, total soluble solids, lycopene content, titratable acidity, fruit set percentage, fruit weight, and overall yield with the application of 30 µg/mL GA_3 and 75 µg/mL 4-CPA.

In the case of LST-6, applying 30 µg/mL GA_3 and 75 µg/mL 4-CPA also led to notable improvements in various quality attributes. However, the values obtained for LST-6 were statistically comparable to those observed in PVB-4, indicating that both cultivars responded similarly to the PGR application.

The increased number of fruits, fruit set %, fruit weight and yield with GA_3 treatment could be due to enhanced assimilate accumulation in treated plants (Ujjwal *et al.,* 2018). The application of auxins is also known to reduce the abscission of flowers and fruits as it supresses the ABA signalling pathway (main cause for senescence) in tomato. Dalai *et al. (*2015) also reported that applying GA_3 and auxins in cucumber improved

Table 4. Effect of plant growth regulators on yield and quality attributes in Punjab Varkha Bahar-4

Treatment	No. of fruits per plant	Pollen viability(%)	Total soluble solids (˚brix)	Lycopene content (mg/100g)	Titratable acidity%	Fruit set %	Fruit weight (g)	Yield (g/plant)
Conc.of GA3 (µg/mL)								
10	17ij	74.51ji	3.88a	3.48b	0.52ebdacf	41.4gh	59g	1010gfieh
20	19ih	80.42gefd	3.85a	3.78b	0.55bdac	53.33e	61ef	1160gfdeh
30	24ced	83.64cbd	3.94a	3.76b	0.57ba	62.66cb	62ed	1500bdac
Conc. of 4-CPA (µg/mL)								
15	20hg	81.55cefd	3.90a	3.61b	0.53ebdac	63.33cb	57h	1150gfdeh
45	22feg	82.92cebd	3.92a	3.74b	0.56bac	65.00b	60gf	1330bdec
75	26cb	84.05cb	3.98a	3.81b	0.63a	70a	64cb	1670ba
Control	15kj	73.13ji	3.90a	3.41b	0.50ebdagcf	35j	54i	820kijh

Values represented by same letters are not significantly different as per Tukey's test (P<0.001). Values are the mean values of different stages of development.

Table 5. Effect of plant growth regulators on yield and quality attributes in LST-6

Treatment	No. of fruits per plant	Pollen viability (%)	Total soluble solids (˚brix)	Lycopene content (mg/100g)	Titratable acidity%	Fruit set %	Fruit weight (g)	Yield (g/plant)
Conc.of GA3 (µg/mL)								
10	13kl	76.58jih	3.36a	3.29b	0.40ebdgcf	39.11ih	45mn	590kj
20	16j	98.68a	3.46a	3.34b	0.42ebdgcf	44.42gf	45.98mL	740kij
30	26cb	48.42m	3.5a	3.65b	0.48ebdagcf	58.79d	47.58k	1280fdec
Conc. of 4-CPA (µg/mL)								
15	20.66fhg	52.18l	3.3a	3.5b	0.46ebdagcf	37.49ji	46.89kl	960gfih
45	25.41cbd	56.27k	3.26a	3.24b	0.44ebdagcf	50.5e	47kl	1040gfieh
75	29.71a	77.53gih	3.54a	3.33b	0.51ebdgcf	62.67cb	49.3j	1460bdac
Control	12l	59.52k	3.2a	3.11b	0.38ebdgcf	22.62k	44a	530k

plant metabolic activity, positively influencing the reproductive state. Thus, all these factors can be considered as major reasons for the increased number of fruits per plant, fruit set, fruit weight and yield in tomato plants with the application of GA_3 and 4-CPA. According to Gelmesa *et al. (*2013) application of GA_3 and auxins resulted in an increased rate of assimilate export from leaves, fruit carbon metabolism, and increased protein, carbohydrate, potassium, sugar, starch, phosphorous and organic acids in tissue because of increased activities within kerb cycle and all this ultimately resulted in increased total soluble solids, titratable acidity (%) and lycopene content in tomato. Ali *et al.* (2022) also reported increased total soluble solids by applying GA_3 and 4- CPA.

The application of PGRs helped confer tolerance to tomato plants against elevated temperature mainly by increasing membrane stability, proline content, total phenols, improving photosynthetic efficiency, and reducing lipid peroxidation. PGRs also increased the plant height, leaf area, total soluble sugars, total soluble proteins, pollen viability, fruit set %, fruit weight, total soluble solids, titratable acidity, and lycopene content, ultimately the yield per plant. Both the varieties *i.e.,* LST-6 and PVB-4 exhibited almost similar effects of plant growth regulators at different concentrations. While LST-6 yielded less than PVB-4 in control plants, the outcomes were statistically comparable with the use of plant growth regulators. There was a notable increase of 63.69% in LST-6 yield and 50.8% in PVB-4 yield compared to their respective control groups. The application of 75 µg/mL 4-CPA followed by 30 µg/mL GA_3 was found to be the most effective method for enhancing thermotolerance.

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